EXHIBIT 7

PART 1 OF 2

US006646243B2

(12) **United States Patent**
Pirrung et al.

(10) **Patent No.: US 6,646,243 B2**
(45) **Date of Patent: Nov. 11, 2003**

(54) **NUCLEIC ACID READING AND ANALYSIS SYSTEM**

(75) Inventors: **Michael C. Pirrung**, Durham, NC (US); **J. Leighton Read**, Palo Alto, CA (US); **Stephen P. A. Fodor**, Palo Alto, CA (US); **Lubert Stryer**, Stanford, CA (US)

(73) Assignee: **Affymetrix, Inc.**, Santa Clara, CA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 18 days.

(21) Appl. No.: **10/098,203**

(22) Filed: **Mar. 15, 2002**

(65) **Prior Publication Data**

US 2003/0013100 A1 Jan. 16, 2003

Related U.S. Application Data

(63) Continuation of application No. 09/690,191, filed on Oct. 16, 2000, now Pat. No. 6,403,957, which is a continuation of application No. 09/129,470, filed on Aug. 4, 1998, now Pat. No. 6,329,143, which is a continuation of application No. 08/456,598, filed on Jun. 1, 1995, now Pat. No. 6,225,625, which is a division of application No. 07/954,646, filed on Sep. 30, 1992, now Pat. No. 5,445,934, which is a division of application No. 07/850,356, filed on Mar. 12, 1992, now Pat. No. 5,405,783, which is a division of application No. 07/492,462, filed on Mar. 7, 1990, now Pat. No. 5,143,854, which is a continuation-in-part of application No. 07/362,901, filed on Jun. 7, 1989, now abandoned.

(51) **Int. Cl.**[7] **H01J 40/00**; C12Q 1/68; C07H 21/02

(52) **U.S. Cl.** **250/200**; 250/302; 250/458.1; 250/559.38; 435/6; 435/7.1; 536/22.1; 536/25.32

(58) **Field of Search** 250/200, 302, 250/458.1, 559.38; 435/6, 7.1; 536/22.1, 25.32

(56) **References Cited**

U.S. PATENT DOCUMENTS

3,730,844	A	5/1973	Gilham et al.	195/103.5 R
3,849,137	A	11/1974	Barzynski et al.	96/97
3,862,056	A	1/1975	Hartman	252/511

(List continued on next page.)

FOREIGN PATENT DOCUMENTS

CA	1284931	6/1991
DE	2242394	3/1974
DE	3440141	5/1986
DE	3505287	3/1988
EP	046 083	2/1982
EP	088 636	9/1983
EP	103 197	3/1984

(List continued on next page.)

OTHER PUBLICATIONS

Bains, "Mixed hybridization and conventional strategies for DNA sequencing," *Genetic Analysis Techniques and Applications*, 10(3–4):84–94 (1993).
Dovichi et al., "DNA sequencing by capillary electrophoresis," *Electrophoresis*, 18:2393–2399 (1997).
Effenhauser et al., "Integrated chip–based capillary electrophoresis," *Electrophoresis*, 18:2203–2213 (1997).

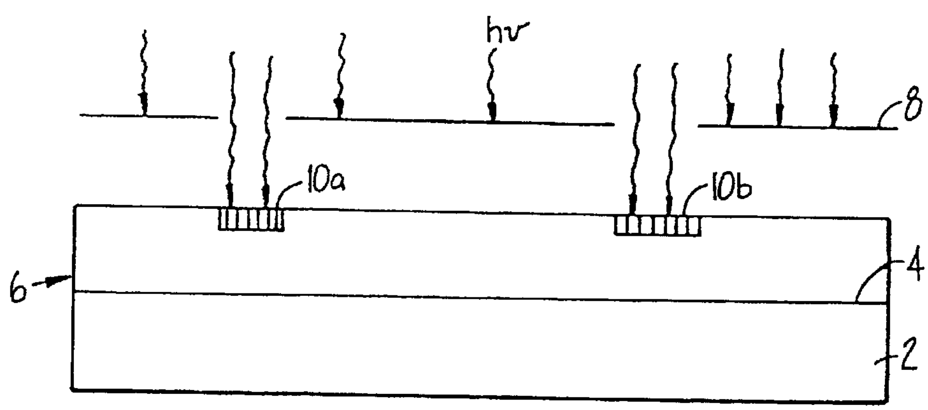
(List continued on next page.)

Primary Examiner—Jezia Riley
(74) *Attorney, Agent, or Firm*—Philip McGarrigle

(57) **ABSTRACT**

A method and apparatus for preparation of a substrate containing a plurality of sequences. Photoremovable groups are attached to a surface of a substrate. Selected regions of the substrate are exposed to light so as to activate the selected areas. A monomer, also containing a photoremovable group, is provided to the substrate to bind at the selected areas. The process is repeated using a variety of monomers such as amino acids until sequences of a desired length are obtained. Detection methods and apparatus are also disclosed.

53 Claims, 22 Drawing Sheets



U.S. PATENT DOCUMENTS

3,939,350 A 2/1976 Kronick et al. 250/365
4,072,576 A 2/1978 Arwin et al. .... 195/103.5 R
4,121,222 A 10/1978 Diebold et al. 347/7
4,180,739 A 12/1979 Abu-Shumays .... 250/461 R
4,216,245 A 8/1980 Johnson 427/2.13
4,238,757 A 12/1980 Schenck 357/25
4,269,933 A 5/1981 Pazos 430/291
4,314,821 A 2/1982 Rice .... 23/230 B
4,327,073 A 4/1982 Huang 424/1
4,339,528 A 7/1982 Goldman 430/323
4,342,905 A 8/1982 Fujii et al. 250/201
4,373,071 A 2/1983 Itakura 525/375
4,395,486 A 7/1983 Wilson et al. 435/6
4,405,771 A 9/1983 Jagur 528/266
4,444,878 A 4/1984 Paulus 435/7
4,444,892 A 4/1984 Malmros 436/528
4,448,534 A 5/1984 Wertz et al. 356/435
4,455,089 A 6/1984 Yeung et al.
4,458,066 A 7/1984 Caruthers et al. 536/27
4,483,920 A 11/1984 Gillespie et al. 435/6
4,498,774 A 2/1985 Yeung et al.
4,500,707 A 2/1985 Caruthers et al. 536/27
4,500,919 A 2/1985 Schreiber 358/78
4,516,833 A 5/1985 Fusek 350/162.12
4,517,338 A 5/1985 Urdea et al. 525/54.11
4,533,682 A 8/1985 Tortorello et al. 523/414
4,537,861 A 8/1985 Elings et al. 436/518
4,542,102 A 9/1985 Dattagupta et al. 435/6
4,555,490 A 11/1985 Merril 436/86
4,555,491 A 11/1985 Spurlin et al.
4,556,643 A 12/1985 Paau et al. 435/5
4,562,157 A 12/1985 Lowe et al. 435/291
4,563,419 A 1/1986 Ranki et al. 435/6
4,569,967 A 2/1986 Kornreich et al. 525/54.11
4,575,433 A 3/1986 Spurlin et al.
4,580,895 A 4/1986 Patel 356/39
4,584,277 A 4/1986 Ullman 436/501
4,588,682 A 5/1986 Groet et al. 435/6
4,591,570 A 5/1986 Chang 435/7.24
4,598,049 A 7/1986 Zelinka et al. 422/116
4,613,566 A 9/1986 Potter 435/6
4,624,915 A 11/1986 Schindler et al. 435/4
4,626,684 A 12/1986 Landa 250/328
4,631,211 A 12/1986 Houghten 428/35
4,634,574 A 1/1987 Spurlin et al.
4,637,861 A 1/1987 Krull et al. .... 204/1 T
4,656,127 A 4/1987 Mundy 435/6
4,670,380 A 6/1987 Dattagupta 435/6
4,677,054 A 6/1987 White et al. 435/6
4,681,859 A 7/1987 Kramer 436/501
4,683,195 A 7/1987 Mullis et al. 435/6
4,683,202 A 7/1987 Mullis 435/91
4,689,405 A 8/1987 Frank et al. 536/27
4,704,353 A 11/1987 Humphries et al. 435/4
4,711,955 A 12/1987 Ward et al. 536/29
4,713,326 A 12/1987 Dattagupta et al. 435/6
4,713,347 A 12/1987 Mitchell et al. 436/501
4,715,413 A 12/1987 Backlund et al. 141/94
4,716,106 A 12/1987 Chiswell 435/6
4,719,179 A 1/1988 Barany 435/172.1
4,719,615 A 1/1988 Feyrer et al. 369/284
4,722,906 A 2/1988 Guire 436/501
4,728,502 A 3/1988 Hamill 422/116
4,728,591 A 3/1988 Clark et al. 430/5
4,731,325 A 3/1988 Palva et al. 435/6
4,737,344 A 4/1988 Koizumi et al. 422/100
4,755,458 A 7/1988 Rabbani et al. 435/5
4,762,881 A 8/1988 Kauer 525/54.11
4,766,062 A 8/1988 Diamond et al. 435/6
4,767,700 A 8/1988 Wallace 435/6
4,777,019 A 10/1988 Dandekar 422/68
4,780,504 A 10/1988 Buendia et al. 525/54.11
4,786,170 A 11/1988 Groebler 356/318
4,786,684 A 11/1988 Glass 525/54.1
4,794,150 A 12/1988 Steel 525/54.11
4,808,508 A 2/1989 Platzer 430/143
4,810,869 A 3/1989 Yabe et al. 250/201
4,811,062 A 3/1989 Tabata et al. 356/152
4,811,218 A 3/1989 Hunkapiller et al.
4,812,512 A 3/1989 Buendia et al. 525/54.11
4,820,630 A 4/1989 Taub 435/5
4,822,566 A 4/1989 Newman 422/68
4,833,092 A 5/1989 Geysen 436/501
4,844,617 A 7/1989 Kelderman et al. 356/372
4,846,552 A 7/1989 Veldkamp et al. 350/162.2
4,849,513 A 7/1989 Smith et al. 536/27
4,855,225 A 8/1989 Fung et al. 435/6
4,865,990 A 9/1989 Stead et al. 435/803
4,868,103 A 9/1989 Stavrianopoulos et al. 435/5
4,874,500 A 10/1989 Madou et al. 204/412
4,875,169 A 10/1989 Synovec et al.
4,877,745 A 10/1989 Hayes et al. 436/166
4,886,741 A 12/1989 Schwartz 435/5
4,888,278 A 12/1989 Singer et al. 435/6
4,921,348 A 5/1990 Yeung et al.
4,921,805 A 5/1990 Gebeyehu et al. 435/270
4,923,901 A 5/1990 Koester et al. 521/53
4,925,785 A 5/1990 Wang et al. 435/6
4,931,384 A 6/1990 Layton et al. 435/7.31
4,946,942 A 8/1990 Fuller et al. 530/335
4,965,188 A 10/1990 Mullis et al. 435/6
4,973,493 A 11/1990 Guire 427/2
4,979,959 A 12/1990 Guire 623/66
4,981,783 A 1/1991 Augenlicht 435/6
4,981,985 A 1/1991 Kaplan et al. 556/50
4,984,100 A 1/1991 Takayama et al. 360/49
4,984,857 A 1/1991 Yeung et al.
4,987,065 A 1/1991 Stavrianopoulos et al. 435/5
4,988,617 A 1/1991 Landegren et al. 435/6
4,992,383 A 2/1991 Farnsworth 436/89
4,994,373 A 2/1991 Stavrianopoulos et al. 435/6
5,002,867 A 3/1991 Macevicz 435/6
5,006,464 A 4/1991 Chu et al. 435/7.1
5,011,770 A 4/1991 Kung et al. 435/6
5,013,669 A 5/1991 Peters, Jr. et al. 436/518
5,021,550 A 6/1991 Zeiger 530/334
5,026,773 A 6/1991 Steel 525/54.11
5,026,840 A 6/1991 Dattagupta et al. 536/27
5,028,525 A 7/1991 Gray et al. 435/6
5,028,545 A 7/1991 Soini 436/501
5,037,882 A 8/1991 Steel 525/54.11
5,043,265 A 8/1991 Tanke et al. 435/6
5,047,524 A 9/1991 Andrus et al. 536/27
5,064,754 A 11/1991 Mills 435/6
5,077,085 A 12/1991 Schnur et al. 427/98
5,077,210 A 12/1991 Eigler et al. 435/176
5,079,600 A 1/1992 Schnur et al. 357/4
5,081,584 A 1/1992 Omichinski et al. 364/497
5,082,830 A 1/1992 Brakel et al. 514/44
5,091,652 A 2/1992 Mathies et al. 250/458.1
5,096,807 A 3/1992 Leaback 435/6
5,100,626 A 3/1992 Levin 422/100
5,100,777 A 3/1992 Chang 435/7.24
5,112,962 A 5/1992 Letsinger et al. 536/27
5,141,813 A 8/1992 Nelson 428/402
5,143,854 A 9/1992 Pirrung et al. 436/518
5,149,625 A 9/1992 Church et al. 435/6
5,153,319 A 10/1992 Caruthers et al. 536/27
5,164,319 A 11/1992 Hafeman et al. 435/287.1

5,171,534 A	12/1992	Smith et al.	
5,171,695 A	12/1992	Ekins	436/501
5,188,963 A	2/1993	Stapleton	435/288.3
5,192,407 A	3/1993	Yeung et al.	
5,192,980 A	3/1993	Dixon et al.	356/326
5,200,051 A	4/1993	Cozzette et al.	204/403
5,202,231 A	4/1993	Drmanac et al.	435/6
5,206,137 A	4/1993	Ip et al.	435/6
5,215,882 A	6/1993	Bahl et al.	435/6
5,215,889 A	6/1993	Schultz	435/41
5,219,726 A	6/1993	Evans	435/6
5,225,326 A	7/1993	Bresser et al.	435/6
5,232,829 A	8/1993	Longiaru et al.	435/6
5,235,028 A	8/1993	Barany et al.	528/335
5,242,794 A	9/1993	Whiteley et al.	
5,242,974 A	9/1993	Holmes	525/54.11
5,252,743 A	10/1993	Barrett et al.	548/303.7
5,256,549 A	10/1993	Urdea et al.	435/91
5,258,506 A	11/1993	Urdea et al.	536/23.1
5,306,641 A	4/1994	Saccocio	436/85
5,310,893 A	5/1994	Erlich et al.	536/24.31
5,324,633 A	6/1994	Fodor et al.	435/6
5,328,824 A	7/1994	Ward et al.	435/6
5,348,855 A	9/1994	Dattagupta et al.	435/6
5,384,261 A	1/1995	Winkler et al.	436/518
5,405,783 A	4/1995	Pirrung et al.	436/518
5,424,186 A	6/1995	Fodor et al.	435/6
5,424,188 A	6/1995	Schneider et al.	435/6
5,432,099 A	7/1995	Ekins	436/518
5,436,327 A	7/1995	Southern et al.	536/25.34
5,445,934 A	8/1995	Fodor et al.	435/6
5,447,841 A	9/1995	Gray et al.	435/6
5,474,796 A	12/1995	Brennan	427/2.13
5,486,452 A	1/1996	Gordon et al.	435/5
5,489,507 A	2/1996	Chehab	435/6
5,489,678 A	2/1996	Fodor et al.	536/22.1
5,492,806 A	2/1996	Drmanac et al.	435/5
5,494,810 A	2/1996	Barany et al.	435/91.52
5,510,270 A	4/1996	Fodor et al.	436/518
5,521,065 A	5/1996	Whiteley et al.	
5,525,464 A	6/1996	Drmanac et al.	435/6
5,527,681 A	6/1996	Holmes	435/6
5,552,270 A	9/1996	Khrapko et al.	435/6
5,556,961 A	9/1996	Foote et al.	536/27.1
5,561,071 A	10/1996	Hollenberg et al.	437/1
5,569,584 A	10/1996	Augenlicht	435/6
5,571,639 A	11/1996	Hubbell et al.	430/5
5,593,839 A	1/1997	Hubbell et al.	435/6
5,599,720 A	2/1997	Ekins	436/501
5,604,099 A	2/1997	Erlich et al.	435/6
5,643,728 A	7/1997	Slater et al.	435/6
5,653,939 A	8/1997	Hollis et al.	422/50
5,667,667 A	9/1997	Southern	205/687
5,667,972 A	9/1997	Drmanac et al.	435/6
5,695,940 A	12/1997	Drmanac et al.	435/6
5,698,393 A	12/1997	Macioszek et al.	435/5
5,700,637 A	12/1997	Southern	435/6
5,707,806 A	1/1998	Shuber	435/6
5,744,305 A	4/1998	Fodor et al.	435/6
5,776,737 A	7/1998	Dunn	435/91.1
5,777,888 A	7/1998	Rine et al.	364/496
5,800,992 A	9/1998	Fodor et al.	435/6
5,807,522 A	9/1998	Brown et al.	422/50
5,830,645 A	11/1998	Pinkel et al.	435/6
5,843,767 A	12/1998	Beattie	435/287.1
5,846,708 A	12/1998	Hollis et al.	435/6
5,869,237 A	2/1999	Ward et al.	435/6
5,871,697 A	2/1999	Rothberg et al.	422/68.1
5,972,619 A	10/1999	Drmanac et al.	435/6
6,018,041 A	1/2000	Drmanac et al.	536/24.3
6,025,136 A	2/2000	Drmanac	435/6
6,040,166 A	3/2000	Erlich et al.	435/194
6,054,270 A	4/2000	Southern	435/6
6,200,748 B1	3/2001	Smith et al.	

FOREIGN PATENT DOCUMENTS

EP	127 438	12/1984
EP	063 810	3/1986
EP	174 879	3/1986
EP	194 132	9/1986
EP	228 075	7/1987
EP	245 662	11/1987
EP	268 237	5/1988
EP	130 523	6/1988
EP	281 927	9/1988
EP	228 310	10/1988
EP	288 310	10/1988
EP	304 202	2/1989
EP	307 476	3/1989
EP	319 012	6/1989
EP	328 256	8/1989
EP	333 561	9/1989
EP	337 498	10/1989
EP	386 229	4/1990
EP	373 203	6/1990
EP	392 546	10/1990
EP	142 299	12/1990
EP	173 339	1/1992
EP	171 150	3/1992
EP	237 362	3/1992
EP	185 547	6/1992
EP	260 634	6/1992
EP	232 967	4/1993
EP	235 726	5/1993
EP	476 014	8/1994
EP	225 807	10/1994
EP	717 113	6/1996
EP	721 016	7/1996
EP	535 242	9/1997
EP	848 067	6/1998
EP	619 321	1/1999
FR	2559783	3/1988
GB	2156074	3/1988
GB	2196476	4/1988
GB	8810400	5/1988
GB	2233654	1/1991
GB	2248840	9/1992
JP	49-110601	10/1974
JP	60-248669	12/1985
JP	63-084499	4/1988
JP	63-223557	9/1988
JP	1-233447	9/1989
NO	P 913186	8/1991
WO	WO 84/03151	8/1984
WO	WO 84/03564	9/1984
WO	WO 85/01051	3/1985
WO	WO 86/00991	2/1986
WO	WO 86/06487	11/1986
WO	WO 88/01058	2/1988
WO	WO 88/04777	6/1988
WO	WO 89/05616	6/1989
WO	WO 89/08834	9/1989
WO	WO 89/10977	11/1989
WO	WO 89/11548	11/1989
WO	WO 89/12819	12/1989
WO	WO 90/00626	1/1990
WO	WO 90/00887	2/1990
WO	WO 90/15070	2/1990
WO	WO 90/03382	4/1990
WO	WO 90/04652	5/1990
WO	WO 90/05789	5/1990
WO	WO 90/07582	7/1990

WO	WO 91/00868	1/1991
WO	WO 91/04266	4/1991
WO	WO 91/07087	5/1991
WO	WO 92/16655	1/1992
WO	WO 92/10092	6/1992
WO	WO 92/10588	6/1992
WO	WO 93/02992	2/1993
WO	WO 88/01302	3/1993
WO	WO 93/09668	5/1993
WO	WO 93/11262	6/1993
WO	WO 93/17126	9/1993
WO	WO 93/22456	11/1993
WO	WO 93/22480	11/1993
WO	WO 95/00530	1/1995
WO	WO 95/11995	5/1995
WO	WO 95/33846	12/1995
WO	WO 96/23078	8/1996
WO	WO 97/10365	3/1997
WO	WO 97/17317	5/1997
WO	WO 97/19410	5/1997
WO	WO 97/27317	7/1997
WO	WO 97/29212	8/1997
WO	WO 97/31256	8/1997
WO	WO 97/45559	12/1997
WO	WO 98/03673	1/1998
WO	WO 98/31836	7/1998
YU	P 570/87	4/1987
YU	18617/87	9/1987

OTHER PUBLICATIONS

Lamture et al., "Direct detecton of nucleic acid hybridization on the surface of a charge coupled device," *Nucleic Acids Research* 22(11):2121–2125 (1994).

Noble, "DNA sequencing on a chip," *Analytical Chemistry*, 67(5):201–204 1995).

O'Donnell–Maloney et al., "Microfabrication and array technologies for DNA sequencing and diagnostics," *Genetic Analysis: Biomolecular Engineering*, 13:151–157 (1996).

Pevzner et al., "Improved chips for sequencing by hybridization," *J. Biomolecular Structure & Dynamics*, 9(2):399–410 (1991).

Smith et al., "Fluorescence detection in automated DNA sequence analysis", *Nature*, 321:674–679 (1986).

Brownlee & Compton, Automated instrumentation for analytical capillary electrophoresis.

Yung–Fong Cheng, et al., Charge–Coupled Device Fluorescence Detection for Capillary–Zone Electrophoresis (CCD–CZE), Applied Spectroscopy, vol. 44, No. 5, 1990.

Kobayashi, et al., Photodiode Array Detection in High–Performance capillary Electrophoresis, Journal of Chromatography, vol. 480, 1989, pp. 179–184, Elsevier Science Publishers, Amsterdam, The Netherlands.

Epperson, et al., Molecular Fluorescence Measurements with a Charge–Coupled Device Detector, Anal. Chem., vol. 61, 1989, pp. 282–285.

Epperson, et al., Binning Spectral Images in a Charge–Coupled Device, Anal. Chem., vol. 61, 1989, pp. 1513–1519.

Epperson, et al., Applications of Charge Transfer Devices in Spectroscopy, Anal. Chem., vol. 60, 1988, pp. 327–335.

Sweedler, et al., High Performance, Anal. Chem., vol. 60, 1988, pp. 282–291.

Sweedler, et al., Fluorescence Detection in Capillary Zone Electrophoresis Using a Charge–Coupled Device with Time–Delayed Integration, Anal. Chem., vol. 63, 1991, pp. 496–502.

Sequencing by Hybridization Workshop, listing of participants and workshop presentation summaries, from workshop held Nov. 19–20, 1991.

"A Sequencing Reality Check," *Science*, 242:1245 (1988).

"Affymax raises $25 million to develop high–speed drug discovery system," *Biotechnology News*, 10(3):7–8 (1990).

"Preparation of fluorescent–labeled DNA and its use as a probe in molecular hybridization," *Bioorg Khim*, 12(11):1508–1513 (1986).

Abbott et al., "Manipulation of the Wettability of Surface on the 0.1—to 1 –Micrometer Scale Through Micromachining and Molecular Self–Assembly," *Science*, 257:1380–1382 (1992).

Adams et al., "Complementary DNA Sequencing: Expressed Sequence Tags and Human Genome Project," *Science*, 252(5013):1651–1656 (1991).

Adams et al., "Photolabile Chelators That "Cage" Calcium with Improved Speed of Release and Pre–Photolysis Affinity," *J. Gen. Physiol.*, p. 9a (12/86).

Adams et al., "Biologically Useful Chelators That Take Up Ca2+ upon Illumination," *J. Am. Chem. Soc.*, 111:7957–7968 (1989).

Ajayaghosh et al., "Solid–Phase Synthesis of N–Methyl– and N–Ethylamides of Peptides Using Photolytically Detachable ((3–Nitro–4((alkylamino)methyl)benzamido)methyl) polystyrene Resin," *J.Org.Chem.*, 55(9):2826–2829 (1990).

Ajayaghosh et al., "Solid–phase synthesis of C–terminal peptide amides using a photoremovable α–methylphenacylamido anchoring linkage," *Proc. Ind. Natl. Sci* (*Chem.Sci.*), 100(5):389–396 (1988).

Ajayaghosh et al., "Polymer–supported Solid–phase Synthesis of C–Terminal Peptide N–Methylamides Using a Modified Photoremovable 3–Nitro–4–N–methylaminomethylpolystyrene Support," *Ind.J.Chem.*, 27B:1004–1008 (1988).

Ajayaghosh et al., "Polymer–Supported Synthesis of Protected Peptide Segments on a Photosensitive 0–Nitro(α–Methyl)Bromobenzyl Resin," *Tetrahedron*, 44(21):6661–6666 (1988).

Amit et al., "Photosensitive Protecting Groups of Amino Sugars and Their Use in Glycoside Synthesis. 2–Nitrobenzyloxycarbonylamino and 6–Nitroveratryloxycarbonylamino Derivatives," *J.Org.Chem*, 39(2):192–196 (1974).

Amit et al., "Photosensitive Protecting Groups—A Review," *Israel J. Chem.*, 12(1–2):103–113 (1974).

Anand et al., "A 3.5 genome equivalent multi access YAC library: construction, characterisation, screening and storage," *Nuc. Acids Res.*, 18(8):1951–1956 (1990).

Anderson et al., "Quantitative Filter Hybridisation," chapter 3 from *Nucleic Acid Hybridization a practical approach*, pp. 73–111, Hames et al., eds., IRL Press (1985).

Applied Biosystems, Model 431A Peptide Synthesizer User's manual, Section 2 and 6, (Aug. 15, 1989).

Arnold et al., "A Novel Universal Support for DNA & RNA Synthesis," abstract from *Federation Proceedings*, 43(7):abstract No. 3669 (1984).

Atherton et al., Solid Phase Peptide Synthesis: A Practical Approach, IRL Press, (1989), tbl. of cont., pp. vii–ix.

Augenlicht et al., "Cloning and Screening of Sequences Expressed in a Mouse Colon Tumor," *Cancer Research*, 42:1088–1093 (1982).

Augenlicht et al., "Expression of Cloned Sequences in Biopsies of Human Tissue and in Colonic Carcinoma Cells Induced to Differentiate in Vitro," *Cancer Res.*, 47:6017–6021 (1987).

Bains, W., "Hybridization Methods for DNA Sequencing," *Genomics*, 11(2):294–301 (1991).

Bains et al., "A Novel Method for Nucleic Acid Sequence Determination," *J.Theor.Biol.*, 135:303–307 (1988).

Bains, W., "Alternative Routes Through the Genome," *Biotechnology*, 8:1251–1256 (1988).

Balachander et al., "Functionalized Siloxy–Anchored Monolayers with Exposed Amino, Azido, Bromo, or Cyano Groups," *Tetrahed. Ltrs.*, 29(44):5593–5594 (1988).

Baldwin et al., "New Photolabile Phosphate Protecting Groups," *Tetrahed.*, 46(19):6879–6884 (1990).

Bannwarth et al., "Laboratory Methods, A System for the Simultaneous Chemical synthesis of Different DNA Fragments on Solid Support," *DNA*, 5(5):413–419 (1986).

Bannwarth, W., "Gene Technology: a Challenge for a Chemist," *Chimia*, 41(9):302–317 (1987).

Barany, F., "Genetic disease detection and DNA amplification using cloned thermostable ligase," *PNAS*, 88:189–193 (1991).

Barltrop et al., "Photosensitive Protective Groups," *Chemical Communications*, pp. 822–823 (1966).

Barinaga, M., "Will 'DNA Chip' Speed Genome Initiative," *Science*, 253:1489 (1985).

Bart et al., "Microfabricated Electrohydrodynamic Pumps," *Sensors and Actuators*A21–A23:193–197 (1990).

Bartsh et al., "Cloning of mRNA sequences from the human colon: Preliminary characterisation of defined mRNAs in normal and neoplastic tissues," *Br.J.Can.*, 54:791–798 (1986).

Baum, R., "Fledgling firm targets drug discovery process," *Chem. Eng. News*, p. 10–11 (1990),

Beltz et al., "Isolation of Multigene Families and Determination of Homologies by Filter Hybridization Methods," *Methods in Enzymology*, 100:266–285 (1983).

Benschop, Chem. Abstracts 114(26):256643 (1991).

Bhatia et al., "New Approach To Producing Patterned Biomolecular Assemblies," *J. American Chemical Society*, 114:4432–4433 (1992).

Biorad Chromatography Electrophoresis Immunochemistry Molecular Biology HPLC catalog M 1987 pp. 182.

Blawas et al., "Step–and–Repeat Photopatterning of Protein Features Using Caged–Biotin–BSA: Characterization and Resolution," *Langmuir*, 14(15):4243–4250 (1998).

Blawas, A.S., "Photopatterning of Protein Features using Caged–biotin–Bovine Serum Albumin," dissertion for Ph.D. at Duke University in 1998.

Bos et al., "Amino–acid substirutions at codon 13 of the N–ras oncogene in human acute myeloid leukaemia," *Nature*, 315:726–730 (1985).

Boyle et al., "Differential distribution of long and short interspersed element sequences in the mouse genome: Chromosome karyotyping by fluorescence in situ hybridization," *PNAS*, 87:7757–7761 (1990).

Brock et al., "Rapid fluorescence detection of in situ hybridization with biotinylated bovine herpesvirus–1 DNA probes," *J.Veterinary Diagnostic Invest.*, 1:34–38 (1989).

Burgi et al., "Optimization in Sample Stacking for High–Performance Capillary Electrophoresis," *Anal. Chem.*, 63:2042–2047 (1991).

Cameron et al., "Photogeneration of Organic Bases from o–Nitrobenzyl–Derived Carbamates," *J. Am. Chem. Soc.*, 113:4303–4313 (1991).

Carrano et al., "A High–Resolution, Fluorescence–Based, Semiautomated Method for DNA Fingerprinting," *Genomics*, 4:129–136 (1989).

Caruthers, M.H., "Gene Synthesis Machines: DNA Chemistry and Its Uses," *Science*, 230:281–285 (1985).

Chatterjee et al., "Inducible Alkylation of DNA Using an Oligonucleotide–Quinone Conjugate," *Am. J. Chem. Soc.*, 112:6397–6399 (1990).

Chee et al., "Accessing Genetic Information with High–Density DNA Arrays," *Science*, 274:610–614 (1996).

Chehab et al., "Detection of sicle cell anaemia mutation by colour DNA amplification," *Lancet*, 335:15–17 (1990).

Chehab et al., "Detection of specific DNA Sequences by fluorescence amplification: A color complementation assay," *PNAS*, 86:9178–9182 (1989).

Chetverin et al., "Oligonucleotide Arrays: A New Concepts and Possibilities," *Biotechnology*, 12:1093–1099 (1994).

Church et al., "Multiplex DNA sequencing," *Science*, 240:185–188 (1988).

Church et al., "Genomic Sequencing," *PNAS*, 81:1991–1995 (1984).

Clevite Corp., Piezoelectric Technology, Data for Engineers.

Corbette et al., "Reaction of Nitroso Aromatic with Glyoxylic Acid. A New Path to Hydroxamic Acids," *J. Org. Chem.*, 45:2834–2839 (1980).

Coulson et al., "Toward a physical map of the genome of the nematode *Caenorhabditis elegans*," *PNAS*, 83:7821–7825 (1986).

Craig et al., "Ordering of cosmid clones covering the Herpes simplex virus type 1 (HSV–1) genome: a test case for fingerprinting by hybridization," *Nuc. Acid. Res.*, 18(9):2653–2660 (1990).

Cummings et al., "Photoactivable Fluorophores. 1. Synthesis and Photoactivation of o–Nitrobenzyl–Quenched Fluorescent Carbamates," *Tetrahederon Letters*, 29(1):65–68 (1988).

Dattagupta et al., "Rapid Identification of Microorganisms by Nucleic Acid Hybridization after Labelling the Test Sample," *Anal. Biochem.*, 177:85–89 (1989).

Dattagupta et al., "Nucleic Acid Hybridization: a Rapid Method for the Diagnosis of Infectious Diseases," *Perspectives in Antiinfective Therapy*, eds. Jackson et al., pp. 241–247 (1988).

Dower et al., "The Search for Molecular Diversity (II): Recombinant and Synthetic Randomized Peptide Libraries," *Ann. Rep. Med. Chem.*, 26:271–280 (1991).

Diggelmann, "Investigating the VLSIPS synthesis process," Sep. 9, 1994.

Di Mauro et al., "DNA Technology in Chip Construction," *Adv. Mater.*, 5(5):384–386 (1993).

Drmanac et al., "An Algorithm for the DNA Sequence Generation from k–Tuple Word Contents of the Minimal Number of Random Fragments," *J. Biomol.Struct. Dyn.*, 8(5):1085–1102 (1991).

Drmanac et al., "Partial Sequencing by Oligo–Hybridization Concept and Applications in Genome Analysis," 1st Int. Conf. Electrophor., Supercomp., Hum. Genome pp. 60–74 (1990).

Drmanac et al., "Sequencing by Oligonucleotide Hybridization: A Promising Framework in Decoding of the Genome Program?," 1st Int. Conf. Electrophor., Supercomp., Hum. Genome pp. 47–59 (1990).

Drmanac et al., "Laboratory Methods, Reliable Hybridization of Oligonucleotides as Short as Six Nucleotides," *DNA and Cell Biol.*, 9(7):527–534 (1990).

Drmanac et al., "Sequencing of Megabase Plus DNA by Hybridization: theory of the Method," *Genomics*, 4:114–128 (1989).

Drmanac et al., "Sequencing of Megabase Plus DNA by Hybridization: Theory of the Method," abstract of presentation given at Cold Spring Harbor Symposium on Genome Mapping and Sequencing, Apr. 22, 1988 thru May 1, 1988.

Dulcey et al. "Deep UV Photochemistry of Chemisorbed Monolayers: Patterned Coplanar Assemblies," *Science*, 252:551–554 (1991).

Duncan et al., "Affinity Chromatography of a Sequence–Specific DNA Binding Protein Using Teflon–Linked Oligonucleotides," *Analytical Biochemistry*, 169:104–108 (1988).

Effenhauser et al., "Glass Chips High–Speed Capillary Electrophoresis Separation with Submicrometer Plate Heights," Anal. Chem., 65:2637–2642 (1993).

Effenhauser et al., "High–Speed Separation of Antisense Oligonucleotides on a Micromachined Capillary Electrophoresis Device," *Anal. Chem.*, 66:2949–2953 (1994).

Ekins et al., "High Specific Activity Chemiluminescent and Fluorescent Markers: their Potential Application to High Sensitivity and 'Multi–analyte' Immunoassays," *J. Bioluminescence Chemiluminescence*, 4:59–78 (1989).

Ekins et al., "Development of Microspot Multi–Analyte Ratiometric Immunoassay Using dual Fluorescent–Labelled Antibodies," *Anal. Chemica Acta*, 227:73–96 (1989).

Ekins et al., "Multianalyte Microspot Immunoassay–Microanalytical 'Compact Disk' of the Future," *Clin. Chem.*, 37(11):1955–1967 (1991).

Ekins et al., R.P., "Multi–Analyte immunoassay*," *J. Pharmaceut, Biomedical Analysis*, 7(2):155–168 (1989).

Ekins et al., "Fluorescence Spectroscopy and its Application to a New Generation of High Sensitivity, Multi–Microspot, Multianalyte, Immunoassay," *Clin. Chim. Acta.*, 194:91–114 (1990).

Elder, J.K., "Analysis of DNA Oligonucleotide Hybridization Data by Maximum Entropy," in *Maximum Entropy and Bayesian Methods*, eds. Mohammad–Djafari amd Demoment, Kluwer, Dordrecht, pp. 363–371 (1992).

Ellis, R.W., "The Applications of Synthetic Oligonucleotides to Molecular Biology," *Pharmaceutical Research*, 3(4):195–207 (1986).

Evans et al., "Microfabrication for Automation of Molecular processes in Human Genome Analysis," *Clin. Chem.*, 41(11):1681 (1995).

Evans et al., "Physical mapping complex genomes by cosmid multiplex analysis," *PNAS*, 86:5030–5034 (1989).

Ezaki et al., "Small–Scale DNA Preparation for Rapid Genetic Identification of Campylobacter Species without Radioisotope," *Microbiol. Immunology*, 32(2):141–150 (1988).

Fan et al., "Mapping small DNA sequences by fluorescence in situ hybridization directly on banded metaphase chromosomes," PNAS, 87(16):6223–6227 (1990).

Fan et al., "Micromachining of Capillary Electrophoresis Injectors and Separators on Glass Chips and Evaluation of Flow at Capillary Intersections," Anal. Chem., 66:177–184 (1994).

Feinberg et al., Addendum to "A technique for Radiolabeling DNA Restriction Endonuclease Fragments to High Specific Activity," *Anal. Biochem.*, 137:266–267 (1984).

Fettinger et al., "Stacked modules for micro flow systems in chemical analysis: concept and studies using an enlarged model," *Sensors and Actuators*, B17:19–25 (1993).

Flanders et al., "A new interferometric alignment technique," *Appl. Phys. Ltrs.*, 31(7):426–429 (1977).

Fodor et al., "Multiplexed biochemical assays with biological chips," *Nature*, 364:555–556 (1993).

Fodor et al., "Light–directed, Spatially Addressable Parallel Chemical Synthesis," *Science*, 251:767–773 (1991).

Forman et al., "Thermodynamics of Duplex Formation and Mismatch Discrimination on Photolithographically Synthesized Oligonucleotide Arrays," chapter 13pp. 206–228 from *Molecular Modeling of Nucleic Acids*, ACS Symposium Series 682, Apr. 13–17, 1997, Leontis et al., eds.

Frank et al., "Simultaneous Multiple Peptide Synthesis Under Continuous flow Conditions on Cellulose Paper Discs as Segmental Solid Supports," *Tetrahedron*, 44(19):6031–6040 (1988).

Frank et al., "Automation of DNA Sequencing Reactions and Related Techniques: A Workstation for Micromanipulation of Liquids," *Bio/Technology*, 6:1211–1212 (1988).

Frank et al., "Simultaneous Synthesis and Biological Applications of DNA Fragments: An Efficient and Complete Methodology," *Methods in Enzymology*, 154:221–250 (1987).

Fuhr et al., "Travelling wave–driven microfabricated electrohydrodynamic pumps for liquids," *J. Micromech. Microeng.*, 4:217–226 (1994).

Fuller et al., "Urethane–Protected Amino Acid N–Carboxy Anhydrides and Their Use in Peptide Synthesis," *J. Amer. Chem. Soc.*, 112(20):7414–7416 (1990).

Furka et al. "General method for rapid synthesis of multicomponent peptide mixtures," *Int. J. Peptide Protein Res.*, 37:487–493 (1991).

Furka et al., "Cornucopia of Peptides by Synthesis," 14th Int.Congress of Biochem. abst.# FR:013, Jul. 10–15, 1988, Prague, Czechoslovakia.

Furka et al., "More Peptides by Less Labour," abst. 288, Int. Symp. Med. Chem., Budapest Hungary Aug. 15–19, 1988.

Gait, eds., pp. 1–115 from *Oligonucleotide Synthesis: A Practical Approach*, IRL Press, (1984).

Gazard et al., "Lithographic Technique Using Radiation–Induced Grafting of Acrylic Acid into Poly(Methyl Methacrylate) Films," *Polymer Engineering and Science*, 20(16):1069–1072 (1980).

Gergen et al., "Filter replicas and permanent collections of recombinant DNA plasmids," *Nuc.Acids Res.*, 7(8):2115–2137 (1979).

Getzoff et al., "Mechanisms of Antibody Binding to a Protein," *Science*, 235:1191–1196 (1987).

Geysen et al., "Strategies for epitope analysis using peptide synthesis," *J. Immunol. Meth.*, 102:259–274 (1987).

Geysen et al., "Use of peptide synthesis to probe viral antigens for epitopes to a resolution of a single amino acid," *PNAS*, 81:3998–4002 (1984).

Geysen et al., "A synthetic strategy for epitope mapping," from Peptides:Chem. & Biol., Proc. of 10th Am. Peptide Symp., May 23–28, 1987, pp. 519–523, (1987).

Geysen et al., "Antigen–antibody interactions at the molecular level: adventures in peptide synthesis," *Immunol. Today*, 6(12):364–369 (1985).

Geysen et al., "Cognitive Features of Continuous Antigenic Determinants," from Synthetic Peptides: Approaches to Biological Probes, pp. 19–30, (1989).

Geysen et al., "Chemistry of Antibody Binding to a Protein," *Science*, 235:1184–1190 (1987).

Geysen et al., "The delineation of peptides able to mimic assembled epitopes," 1986 CIBA Symp., pp. 130–149.

Geysen et al., "Cognitive Features of Continuous Antigenic Determinants," *Mol. Recognit.*, 1(1):1–10 (1988).

Geysen et al., "*A Prio Ri* Delineation of a Peptide Which Mimics A Discontinuous Antigenic Determinant," *Mol. Immunol.*, 23(7):709–715 (1986).

Ghosh et al., "Covalent attachment of oligonuleotides to solid supports," *Nuc. Acids Res.*, 15(13):5353–5373 (1987).

Gilon et al., "Backbone Cyclization: A New Method for Conferring Conformational Constraint on Peptides," *Biopolymers*, 31(6):745–750 (1991).

Gingeras et al., "Hybridization properties of immobilized nucleic acids," *Nuc. Acids Res.*, 15(13):5373–5390 (87).

Gummerlock et al., "RAS Enzyme–Linked Immunoblot Assay Discriminates p12 Species: A Technique to Dissect Gene Family Expression," *Anal. Biochem.*, 180:158–168 (1989).

Gurney et al., "Activation of a potassium current by rapid photochemically generated step increases of intracellular calcium in rat sympathetic neurons,"*PNAS*, 84:3496–3500 (1987).

Haase et l., "Detection of Two Viral Genomes in Single Cells by Double–Label Hybridization in Situ and Color Microradiography," *Science*, 227:189–192 (1985).

Hacia et al., "Two color hybridization analysis using high density oligonucleotide arrays and energy transfer dyes," *Nuc. Acids Res.*, 26(16):3865–3866 (1988).

Hack, M.L., "Conics Formed to Make Fluid & Industrial Gas Micromachines," *Genetics Engineering News*, 15(18):1, 29 (1995).

Hagedorn et al., "Pumping of Water Solutions in Microfabricated Electrohydrodynamic System," from Micro Electro Mechanical Systems Conference in Travemunde Germany (1992).

Hames et al., *Nuclear acid hybridization, a practical approach*, cover page and table of contents (1985).

Hanahan et al., "Plasmid Screening at High Colony Density," *Meth. Enzymology*, 100:333–342 (1983).

Hanahan et al., "Plasmid screening at high colony density," *Gene*, 10:63–67 (1980).

Haridasan et al., "Peptide Synthesis using Photolytically Cleavable 2–Nitrobenzyloxycarbonyl Protecting Group." *Proc. Indian Natn. Sci. Adad.*, 53A(6):717–728 (1987).

Harrison et al., "Capillary Electrophoresis and Sample Injection Systems Integrated on a Planar Glass Chip," *Anal. Chem.*, 64:1926–1932 (1992).

Harrison et al., "Micromachining a Minaturized Capillary Electrophoresis–Based Chemical Analysis System on a Chip," *Science*, 261:895–897 (1993).

Harrison et al., "Towards minaturized electrophoresis and chemical analysis systems on silicon: an alternative to chemical sensors*," *Sensors and Actuators*, B10:107–116 (1993).

Harrison et al., "Rapid separation of fluorescein derivatives using a micromachined capillary electrophoresis system," *Analytica Chemica Acta*, 283:361–366 (1993).

Hellberg et al., "Minimum analogue peptide sets (MAPS) for quantitative structure–activity relationships," *Int. J. Peptide Protein Res.*, 37:414–424 (1991).

Hilser et al., "Protein and peptide mobility in capillary zone electrophoresis, A comparison of existing models and further analysis," *J. Chromatography*, 630:329–336 (1993).

Ho et al., "Highly Stable Biosensor Using an Artificial Enzyme," *Anal.Chem.*, 59:536–537 (1987).

Hochgeschwender et al., "Preferential expression of a defined T–cell receptor β–chain gene in hapten–specific cytotoxic T–cell clones," *Nature*, 322:376–378 (1986).

Hodgson et al., "Assays A La Photolithography," *Biotech.*, 9:419 (1991).

Hodgson et al., "Hybridization probe size control: optimized 'oligolabelling'," *Nuc.Acids Res.*, 15(15):6295 (1987).

Hopman et al., "Bi–color detection of two target DNAs by non–radioactive in situ hybridization*," *Histochem.*, 85:1–4 (1986).

Iwamura et al., "1–Pyrenylmethyl Esters, Photolabile Protecting Groups for Carboxlic Acids," *Tetrahedron Ltrs.*, 28(6):679–682 (1987).

Iwamura et al., "1–(α–Diazobenzyl)pyrene: A Reagent for Photolabile and Fluorescent Protection of Carboxyl Groups of Amino Acids and Peptides," *Synlett*, p. 35–36 (1991).

Jacobson., "Effects of Injection Schemes and Column Geometry on the Performance of Microchip Electrophoresis Devices," *Anal. Chem.*, 66:1107–1113 (1994).

Jacobsen et al., "Open Channel Electrochromatography on a Microchip," *Anal. Chem.*, 66:2369–2373 (1994).

Jacobsen et al., "Microchip Capillary Electrophoresis wih an Integrated Postcolumn Reactor" *Anal. Chem.*,66:3472–3476 (1994).

Jacobson et al., "Precolumn Reactions with Electrophoretic Analysis Integrated on a Microchip," *Anal. Chem.*, 66:4127–4132 (1994).

Jacobson et al., "Microfabricated chemical measurement systems," *Nature Medicine*, 1(10):1093–1096 (1995).

Jacobsen et al., "Fused Quartz Substrates for Microchip Electrophoresis," *Anal. Chem.*, 67:2059–2063 (1995).

Jacobson et al., "High–Speed Separations on a Microchip," *Anal. Chem.*, 66:1114–1118 (1994).

Jacobson et al., "Microchip electrophoresis with sample stacking," *Electrophoresis*, 16:481–486 (1995).

Jayakumari, "Peptide synthesis in a triphasic medium catalysed by papain immobilized on a crosslinked polystyrene support," *Indian J. Chemistry*, 28B:514–517 (1990).

Jovin et al., "Luminescence Digital Imaging Microscopy," *Ann. Rev. Biophys. Biophys. Chem.*, 18:271–308 (1989).

Kafatos et al., "Determination of nucleic acid sequence homologies and relative concentrations by a dot hybridization procedure," *Nuc. Acids Res.*, 7(6):1541–1553 (1979).

Kaiser et al., "Peptide and Protein Synthesis by Segment Synthesis–Condensation," *Science*, 243:187–192 (1989).

Kaplan et al., "Photolabile chelators for the rapid photorelease of divalent cations," *PNAS*, 85:6571–6575 (1988).

Karube et al., "Micro–biosensors based on Silicon fabrication technology," chapter 25 from biosensors:Fundamentals and Applications, Turner et al., eds., Oxford Publ., 1987, pp. 471–480 (1987).

Kates et al., "A Novel, Convenient, Three–dimensional Orthogonal Strategy for Solid–Phase Synthesis of Cyclic Peptides 1–3," *Tetrahed. Letters*, 34(10):1549–1552 (1993).

Kerkof et al., "A Procedure for making Simultaneous Determination of the Relative Levels of Gene Transcripts in Tissues or Cells," *Anal. Biochem.*, 188:349–355 (1990).

Khrapko et al., "An Oligonucleotide hybridization approach to DNA sequencing," *FEBS Lett.*, 256(1,2):118–122 (1989).

Khrapko et al., "A method for DNA sequencing by hybridization with aligonucleotide matrix," *DNA Seq. Map.*, 1:375–388 (1991).

Kidd et al., "α_1–Antitrypsin deficiency detection by direct analysis of the mutation on the gene," *Nature*, 304:230–234 (1983).

Kievits et al., "Rapid subchromosomal localization of cosmids by nonradioactive in situ hybridization," *Cytogenetics Cell Genetics*, 53(2–3):134–136 (1990).

Kimura et al., "An Immobilized Enzyme Membrane Fabrication Method using an Ink Jet Nozzle," *Biosensors*, 4:41–52 (1988).

Kimura et al., "An Integrated SOS/FET Multi–Biosensor," *Sensors & Actuators*, 9:373–387 (1986).

Kitazawa et al., "In situ DNA–RNA hybridization using in vivo bromodeoxyuridine–labeled DNA probe," *Histochemistry*, 92:195–199 (1989).

Kleinfeld et al., "Controlled Outgrowth of Dissociated Neurons on Patterned Substrates," *J. Neurosci.*, 8(11):4098–4120 (1988).

Knight, P., "Materials and Methods/Microsequencers for Protein and Oligosaccharides," *Bio/Tech.*, 7:1075–76 (1989).

Kohara et al., "The Physical Map of the Whole *E. coli* Chromosome: Application of a New Strategy for Rapid Analysis and Sorting of a Large Genomic Library," *Cell*, 50:495–508 (1987).

Krile et al., "Multiplex holography with chirp–modulated binary phase–coded reference–beam masks," *Applied Opt.*, 18(1):52–56 (1979).

Labat, I., "Subfragments as an informative characteristic of the DNA molecule—computer simulation," research report submitted to the University of Belgrade College of Natural Sciences and Mathematics, (1988).

Lander et al., "Genomic Mapping by Fingerprinting Random Clones: A Mathematical Analysis," *Genomics*, 2:231–239 (1988).

Lainer et al., "Human Lymphocyte Subpopulations Identified by Using Three–Color Immunofluorescence and Flow Cytometry Analysis: Correlation of Leu–2, Leu–3, Leu–7, Leu 8, Leu–11 Clee Surface Antigen Expression," *Journal of Immunology*, 132(1):151–156 (1984).

Lam et al., "A new type of synthetic peptide library for indentifying ligand–binding activity," *Nature*, 354:82–84 (1991).

Laskey et al., "Messenger RNA prevalence in sea urchin embryos measured with cloned cDNAs," *PNAS*, 77(9):5317–5321 (1980).

Lee et al., "synthesis of a Polymer Surface Containing Covalently Attached Triethoxysilane Functionality: Adhesion to Glass," *Macromolecules*, 21:3353–3356 (1988).

Lehrach et al., "Labelling oligonucleotides to high specific activity (1)," *Nuc. Acids Res.*, 17(12):4605–4610 (89).

Lehrach et al., "Phage Vectors—EMBL Series," *Meth. Enzymology*, 153:103–115 (1987).

Lehrach et al., "Hybridization Fingerprinting in Genome Mapping and Sequencing," *Genome Analysis vol. 1: Genetic and Physical Mapping*, Cold Spring Harbor Laboratory Press, pp. 39–81 (1990).

Levy, M.F., "Preparing Additive Printed Circuits," *IBM Tech. Discl. Bull.*, 9(11):1473 (1967).

Lewin, Benjamin, eds., *Genes*, third edition, John Wiley & Sons, cover page, preface and table of contents, (1987).

Lichter et al., "High–Resolution Mapping of Human Chromosome 11 by in Situ hybridization with Cosmid Clones," *Science*, 247:64–69 (1990).

Lichter et al., "Fluorescence in situ hybridization with Alu and L1 polymerase chain reaction probes for rapid characterization of human chromosomes in hybrid cell lines," *PNAS.*, 87:6634–6638 (1990).

Lichter et al., "Rapid detection of human chromosome 21 aberrations by in situ hybridization," *PNAS*, 85:9664–9668 (1988).

Lichter et al., "Is non–isotopic in situ hybridization finally coming of age," *Nature*, 345:93–94 (1990).

Lieberman et al., "A Light source Smaller Than the Optical Wavelength," *Science*, 247:59–61 (1990).

Lipshutz et al., "Using Oligonucleotide Probe Arrays To Access Genetic Diversity," *Bio/Tech.*, 19(3):442–7 (1995).

Little, P., "Clone maps made simple," *Nature*, 346:611–612 (1990).

Liu et al., "Sequential Injection Analysis in Capillary Format with an Electroosmotic Pump," *Talanta*, 41(11):1903–1910 (1994).

Lockhart et al., "Expression monitoring by hybridization to high–density oligonucleotide arrays," *Nat. Biotech.*, 14:1675–1680 (1996).

Logue et al., "General Approaches to Mask Design for Binary Optics," SPIE, 1052:19–24 (1989).

Loken et al., "three–color Immunofluorescence Analysis of Leu Antigens on Human Peripheral Blood Using Two Lasers on a Fluorescence–Activated Cell Sorter," *Cymoetry*, 5:151–158 (1984).

Love et al., "Screening of λLibrary for Differentially Expressed Genes Using in Vitro Transcripts," *Anal. Biochem.*, 150:429–441 (1985).

Lowe, C.R., "Biosensors," *Trends in Biotech.*, 2:59–65 (1984).

Lowe, C.R., "An Introduction to the Concepts and Technology of Biosensors," *Biosensors*, 1:3–16 (1985).

Lowe, C. R., Biotechnology and Crop Improvement and Protection, BCPC Publications, pp. 131–138 (1986).

Lowe et al., "Solid–Phase Optoelectronic Biosensors," *Methods in Enzymology*, 137:338–347 (1988).

Lowe, C.R., "Biosensors," *Phil. Tran. R. Soc. Lond.*, 324:487–496 (1989).

Lu et al., "Differential screening of murine ascites cDNA libraries by means of in vitro transcripts of cell–cycle–phase–specific cDNA and Digital image processing," *Gene*, 86:185–192 (1990).

Luo, J. et al., "Improving the fidelity of *Thermus thermophilos* DNA ligase," *Nuc.Acids Res.*, 24(14):3071–3078 (1996).

Lysov et al., "A new method for determining the DNA nucleotide sequence by hybridization with oligonucleotides," *Doklady Biochem.*, 303(1–6):436–438 (1989).

Lysov et al., "DNA Sequencing by Oligonucleotide Hybridization," First International Conference on Electrophoresis, Supercomputing and Human Genome, Apr. 10–13, 1990 p. 157.

MacDonald et al., "A Rapid ELISA for Measuring Insulin in a Large Number of Research Samples," *Metabolism*, 38(5):450–452 (1989).

Mairanovsky, V.G., "Electro–Deprotection– Electrochemical Removal of Protecting Groups**," *Angew. Chem. Int. Ed. Engl.*, 15(5):281–292 (1976).

Manz et al., "Miniaturized Total Chemical Analysis Systems: a Novel Concept for Chemical Sensing," *Sensors and Actuators*, B1:244–248 (1990).

Manz et al., "Micromachining of monocrystalline silicon and glass for chemical analysis systems, A look into next century's technology or just a fashionable craze?," *Trends in Analytical Chem.*, 10(5):144–149 (1991).

Manz et al., "Planar chips technology for miniaturization and integration of separation techniques into monitoring systems, Capillary electrophoresis on a chip," *J. Chromatography*, 593:253–258 (1992).

Manz et al., "Planar Chips Technology for Miniaturization of Separation Systems: A Developing Perspective in Chemical Monitoring," chapter 1, 1–64 (1993).

Manz et al., "Electroosmotic pumping and electrophoretic separations for minaturized chemical analysis systems," *J. Micromech. Microeng.*, 4:257–265 (1994).

Masiakowski et al., "Cloning of cDNA sequences of hormone–regulated genes from the MCF–7 human breast cancer cell line," *Nuc. Acids Res.*, 10(24):7895–7903 (1982).

Matsumoto et al., "Preliminary Investigation of Micropumping Based on Electrical Control in Interfacial Tension," *IEEE*, pp. 105–110 (1990).

Matsuzawa et al., "Containment and growth of neuroblastoma cells on chemically patterned substrates," *J. Neurosci. Meth.*, 50:253–260 (1993).

Matthes et al., "Simultaneous rapid chemical synthesis of over one hundred oligonucleotides on a microscale," *EMBO J.*, 3(4):801–805 (1984).

McCray et al., "Properties and Uses of Photoreactive Caged Compounds," *Ann. Rev. Biophys. Biophys. Chem.*, 18:239–270 (1989).

McGall et al., "The Efficiency of Light–Directed Synthesis of DNA Arrays on Glass Substrates," *J. American Chem. Soc.*, 119(22):5081–5090 (1997).

McGillis, VLSI Technology, Sze, eds., Chapter 7, "Lithography," pp. 267–301 (1983).

McMurray, J.S., "Solid Phase Synthesis of a Cyclic Peptide Using Fmoc Chemistry," *Tetrahedron Letters*, 32(52):7679–7682 (1991).

Meinkoth et al., "Review: Hybridization of Nucleic Acids Immobilized on Solid Supports," *Analytical Biochem.*, 138:267–284 (1984).

Melcher et al., "Traveling–Wave Bulk Electroconvection Induced across a Temperature Gradient," *Physics of Fluids*, 10(6):1178–1185 (1967).

Merrifield, R.B., "Solid Phase peptide Synthesis. I. The Synthesis of a Tetrapeptide," *J.Am.Chem.Soc.*, 85:2149–2154 (1963).

Michiels et al., "Molecular approaches to genome analysis: a strategy for the construction of ordered overlapping clone libraries," *CABIOS*, 3(3):203–10 (1987).

Mirzabekov, A.D., "DNA sequencing by hybridization—a megasequencing method and a diagnostic tool?," *TIBTECH*, 12:27–32 (1994).

Miyada et al., "Oligonucleotide Hybridization Techniques," *Meth. Enzymology*, 154:94–107 (1987).

Monaco et al., "Human Genome Linking with Cosmids and Yeast Artificial Chromosomes", abstract from CSHS, p. 50, (1989).

Morita et al., "Direct pattern fabrication on silicone resin by vapor phase electron bean polymerization," *J.Vac.Sci.Technol.*, B1(4):1171–1173 (1983).

Morrison et al., "Solution–Phase Detection of Polynucleotides Using Interacting Fluorescent Labels and Competitive Hybridization," *Anal. Biochem.*, 183:231–244 (1989).

Munegumi et al., "thermal Synthesis of Polypeptides from N–Boc–Amino Acid (Aspartic Acid, β–Aminoglutaric Acid) Anhydrides," *Chem. Letters*, p. 1643–1646 (1988).

Mutter et al., "Impact of Conformation on the Synthetic Strategies for Peptide Sequences," pp. 217–228 from Chemistry of Peptides and Proteins, vol. 1, Proceedings of the Third USSR–FRG Symp., in USSR (1982).

Nakamori et al., "A Simple and Useful Method for Simultaneous Screening of Elevated Levels of Expression of a Variety of Oncogenes in Malignant Cells," *Jpn. J. Cancer Res.*, 79:1311–1317 (1988).

Nederlof et al., "Multiple Fluorescence In Situ Hybridization," *Cytometry*, 11:126–131 (1990).

Nederlof et al., "Three–Color Fluorescence In Situ Hybridization for the Simultaneous Detection of Multiple Nucleic Acid Sequences," *Cytometry*, 10:20–27 (1989).

Nizetic et al., "An improved bacterial colony lysis procedure enables direct DNA hybridisation using short (10, 11 bases) oligonucleotides to cosmids," *Nuc. Acids Res.*, 19(1):182 (1990).

Nizetic et al., "Construction, arraying, and high–density screening of large insert libraries of human chromosomes X and 21: their potential use as reference libraries," *PNAS*, 88:3233–3237 (1991).

Nyborg, W., "Acoustic Streaming," chapter 11 pp. 265–329 from Physical Acoustics, Principles and Methods, Mason, eds., vol. II, part B, Academic Press, New York and London (1965).

Ocvirk et al., "High Performance Liquid Chromatography Partially Integrated onto a Silicon Chip," *Analyt. Meth. Instrumentation*, 2(2):74–82 (1995).

Ohtsuka et al., "Studies on transfer ribonucleic acids and related compounds. IX Ribonucleic oligonucleotide synthesis using a photosensitive O–nitrobenzyl protection at the 2'–hydroxy group," *Nuc.Acids.Res.*, 1(10):1351–1357 (1974).

Olefirowicz et al., "Capillary Electrophoresis for Sampling Single Nerve Cells," *Chimia*, 45(4):106–108 (1991).

Olson et al., "Random–clone strategy for genomic restriction mapping in yeast," *PNAS*, 83:7826–7830 (1986).

Patchornik et al., "Photosensitive Protecting Groups," *J.Am. Chem.Soc.*, 92(21):6333–6335 (1970).

Patent Abstracts of Japan from EPO, Abst. 13:557, JP 1–233 447 (1989).

Pease et al., "Light–generated oligonucleotide arrays for rapid DNA sequence analysis," *PNAS*, 91:5022–26 (1994).

Pevzner, P.A., "DNA Physical Mapping and Alternating Eulerian Cycles in Colored Grapes," *Algorithmica*, 13(1–2):77–105 (1995).

Pevzner et al., "Multiple Filtration and Approximate Pattern Matching," *Algorithmica*, 13(1–2):135–154 (1995).

Pevzner et al., "Generalized Sequence Alignment and Durality," *Adv. Applied Math.*, 14:139–171 (1993).

Pevzner, P.A., "1–Tuple DNA Sequencing: Computer Analysis," *J. Biomol. Struct. Dynam.*, 7(1):63–69 (1989).

Pfahler et al., "Liquid Transport in Micron and Submicron Channels," *Sensors and Actuators*, A21–A23:431–4 (90).

Pfeifer et al., "Genomic Sequencing and Methylation Analysis by Ligation Mediated PCR," *Science*, 246:810–813 (1989).

Pidgeon et al., "Immobilized Artificial Membrane Chromatography: Supports Composed of Membrane Lipids," *Anal. Biochem.*, 176:36–47 (89).

Pillai, V.N., "Photoremovable Protecting Groups in Organic Synthesis," *Synthesis*, pp. 1–26 (1980).

Pillai et al., "3–Nitro–4–Aminomethylbenzoylderivate von Polyethylenglykolen:Eine neue Klasse von Photosensitiven loslichen Polymeren Tragern zur Synthese von C–terminalen Peptidamiden," *Tetrah. ltr.*, #36 p. 3409–3412 (1979).

Pillai et al., "Synthetic Hydrophilic Polymers, Biomedical and Chemical Applications," *Naturwissenschaften*, 68:558–566 (1981).

Pirrung et al., "Proofing of Photolithographic DNA Synthesis with 3',5'–Dimethoxybenzoinyloxycarbonyl–Protected Deoxynucleoside Phosphoramidites," *J. Org. Chem.*, 63(2):241–246 (1998).

Pirrung et al., "Comparison of Methods for Photochemical Phosphoramidite–Based DNA Synthesis," *J. Org. Chem.*, 60:6270–6276 (1995).

Ploax et al., "Cyclization of peptides on a solid support," *Int. J. Peptide Protein Research*, 29:162–169 (1987).

Polsky–Cynkin et al., "Use of DNA Immobilized on Plastic and Agarose Supports to Detect DNA by Sandwich Hybridization," *Clin. Chem.*, 31(9):1428–1443 (1985).

Poustka et al., "Molecular Approaches to Mammalian Genetics," Cold Spring Harbor Symposia on Quantitive Biology, 51:131–139 (1986).

Purushothaman et al., "Synthesis of 4,5–diarylimidazoline–2–thiones and their photoconversion to bis(4,5–diarylimidazol–2–yl) sulphides," *Ind. J. Chem.*, 29B:18–21 (1990).

Quesada et al., "High–Sensitivity DNA Detection with a Laser–Exited Confocal Fluorescence Gel Scanner," *Biotechniques*, 10:616 (1991).

Reichmanis et al., *J. Polymer Sci. Polymer Chem. Edition*, 23:1–8 (1985).

Renz et al., "A colorimetric method for DNA hybridization," *Nuc. Acids Res.*, 12(8):3435–3445 (1984).

Richter et al., "An Electrohydrodynamic Micropump," *IEEE*, pp. 99–104 (1990).

Richter et al., "Electrohydrodynamic Pumping and Flow Measurement," *IEEE*, pp. 271–276 (1991).

Richter et al., "A Micromachined electrohydrodynamic (EHD) pump," *Sensors and Actuators*, A29:159–168 (91).

Robertson et al., "A General and Efficient Route for Chemical Aminoacylation of Transfer RNAs," *J. Am. Chem. Soc.*, 113:2722–2729 (1991).

Rodda et al., "The Antibody Response to Myoglobin–I. Systematic Synthesis of Myglobin Peptides Reveals Location and Substructure of Species–Dependent Continuous Antigenic Determinants," *Mol. Immunol.*, 23(6):603–610 (1986).

Rodgers, R.P., "Data Processing of Immunoassay Results," Manual of Clin. Lab. Immunol., 3rd ed., ch. 15, pp. 82–87 (1986).

Rose, D.J., "Free–solution reactor for post–column fluorescence detection in capillary zone electrophoresis," *J. Chromatography*, 540:343–353 (1991).

Rovero et al., "Synthesis of Cylic Peptides on solid Support," *Tetrahed. Letters*, 32(23):2639–2642 (1991).

Sambrook, Molecular Cloning—A Laboratory Manual, publ. in 1989 (not included).

Saiki et al., "Genetic analysis of amplified DNA with immobilized sequence specific oligonucleotide probes," *PNAS*, 86:6230–6234 (1989).

Saiki et al., "Analysis of enzymatically amplified β–globin and HLA–DQα DNA with Allele–specific oligonucleotide probes," *Nature*, 324:163–166 (1986).

Schafer et al., "DNA fingerprinting using non–radioactive oligonucleotide probes specific for simple repeats," *Nuc. Acids Res.*, 16(19):9344 (1988).

Scharf et al., "HLA class II allelic variation and susceptibility to pemphigus vulgaris," *PNAS*, 85(10):3504–3508 (1988).

Schena et al., "Parallel human genome analysis: Microassay–based expression monitoring of 1000 genes," *PNAS*, 93:10614–10619 (1996).

Schuup et al., "Mechanistic Studies of the Photorearrangement of o–Nitrobenzyl Esters," *J. Photochem.*, 36:85–97 (1987).

Seed, B., "Diazotizable arylamine cellulose papers for the coupling and hybridization of nucleic acids," *Nuc. Acids Res.*, 10(5):1799–1810 (1982).

Seiler et al., "Planar Glass Chips for Capillary Electrophoresis: Repetitive Sample Injection, Quantitation, and Separation Efficiency," *Anal. Chem.*, 65:1481–1488 (1993).

Seller et al., "Electroosmotic Pumping and Valveless Control of Fluid Flow within a Manifold of Capillaries on a Glass Chip," Anal. Chem., 66:3485–3491 (1994).

Semmelhack et al., "Selective Removal of Protecting Groups Using Controlled Potential Electrolysis," *J. Am. Chem. Society*, 94(14):5139–5140 (1972).

Sheldon et al., "Matrix DNA Hybridization," *Chemical Chemistry*, 39(4):718–719 (1993).

Shin et al., "Dehydrooligopeptides. XI. Facile Synthesis of Various Kinds of Dehydrodi– and tripeptides, and Dehydroenkephalins Containing Tyr Residue by Using N–Carboxydehydrotyrosine Anhydride," *Bull. Chem. Soc. Jpn.*, 62:1127–1135 (1989).

Sim et al., "Use of a cDNA Library for Studies on Evolution and Developmental Expression of the Chorion Multigene Families," *Cell*, 18:1303–1316 (1979).

Smith et al., "A Novel Method for Delineating Antigenic Determinants: Peptide Synthesis and Radioimmunoassay Using the Same Solid Support," *Immunochemistry*, 14:565–568 (1977).

Sofia, M.J., "Carbohydrate–based combinatorial libraries," *Molecular Diversity*, 3:75–94 (1998).

Southern et al., "Report on the Sequencing by Hybridization Workshop," *Genomics*, 13:1378–1383 (1992).

Southern et al., "Oligonucleotide hybridisations on glass supports: a novel linker for oligonucleotide synthesis and hybridization properties of oligonucleotides synthesized in situ," *Nuc. Acids Res.*, 20(7):1679–1684 (1992).
Southern et al., "Analyzing and Comparing Nucleic Acid Sequences by Hybridization to Arrays of Oligonucleotides: Evaluation Using Experimental Models," Genomics, 13:1008–10017 (1992).
Southern, E.M., "Detection of Specific Sequences Among DNA Fragments Separated by Gel Electrophoresis," *J. Mol. Biol.*, 98:503–517 (1975).
Stemme et al., "A valveless diffuser/nozzle–based fluid pump," *Sensors and Actuators*, A39:159–167 (1993).
Stryer, L., "DNA Probes and Genes Can Be Synthesized by Automated Solid–Phase Methods," from *Biochemistry*, Third Edition, published by W.H. Freeman & Co., (1988).
Stuber et al., "Synthesis and photolytic cleavage of bovine insulin B22–30 on a nitrobenzoylglycyl–poly (ethylene glycol) support," *Int. J. Peptide Protein Res.*, 22(3):277–283 (1984).
Sundberg et al., "Spatially–Addressable Immobilization of Macromolecules on Solid Supports," *J. Am. Chem. Soc.*, 117(49):12050–12057 (1995).
Swedberg, S.A., "Use of non–ionic and zwitterionic surfactants to enhance selectivity in high–performance capillary electrophoresis, An apparent micellar electrokinetic capillary chromatography mechanism," *J. Chromatography*, 503:449–452 (1990).
Thomas, P.S., "Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose," *PNAS*, 77(9):5201–5205 (1980).
Titus et al., "Texas Red, a Hydrophilic, red–emitting fluorophore for use with fluorescein in dual parameter plow microfluorometric and fluorescence microscopic studies," *J. Immunol. Meth.*, 50:193–204 (1982).
Tkachuk et al., "Detection of *bcr–abl* Fusion in chronic Myelogenous Leukemia by in situ Hybridization," *Science*, 250:559–562 (90).
Trzeciak et al., "Synthesis of 'Head–to–Tail' Cyclized Peptides on Solid Support by FMOC Chemistry," *Tetrahed. Letters*, 33(2):4557–4560 (1992).
Tsien et al., "Control of Cytoplasmic Calcium with Photolabile Tetracarboxylate 2–Nitrobenzhydrol Chelators," *Biophys. J.*, 50:843–853 (1986).
Tsutsumi et al., "Expression of L– and M– Type Pyruvate Kinase in Human Tissues," *Genomics*, 2:86–89 (1988).
Turchinskii et al., "Multiple Hybridization in Genome Analysis, Reaction of Diamines and Bisulfate with Cytosine for Introduction of Nonradioactive labels Into DNA," *Molecular Biology*, 22:1229–1235 (1988).
Turner et al., "Photochemical Activation of Acylated α–Thrombin," *J. Am. Chem. Soc.*, 109:1274–1275 (1987).
Urdea et al., "A novel method for the rapid detection of specific nucleotide sequences in crude biological samples without blotting or radioactivity; application to the analysis of hepatitis B virus in human serum," *Gene*, 61:253–264 (1987).
Urdea et al., "A comparison of non–radioisotopic hybridization assay methods using fluorescent, chemiluminescent and enzyme labeled synthetic oligodeoxyribonucleotide probes," *Nuc. Acids Res.*, 16(11):4937–4956 (1988).
Van der Voort et al., "Design and Use of a Computer Controlled Confocal Microscope for Biological Applications," *Scanning*, 7(2):66–78 (1985).
Van Hijfte et al., "Intramolecular 1,3–Diyl Trapping Reactions. A Formal Total Synthesis of –Coriolin," J. Organic Chemistry, 50:3942–3944 (1985).
Veldkamp, W.B., "Binary optics: the optics technology of the 1990s," CLEO 90, vol. 7, paper # CMG6 (1990).
Verlaan–de Vries et al., "A dot–blot screening procedure for mutated *ras* oncogenes using synthetic oligodeoxynucleotides," *Gene*, 50:313–320 (1986).
Verpoorte et al., "Three–dimensional micro flow manifolds for miniaturized chemical analysis systems," *J. Micromech. Microeng.*, 4:246–256 (1994).
Volkmuth et al., "DNA electrophoresis in microlithographic arrays," *Nature*, 358:600–602 (1992).
Voss et al., "The immobilization of oligonucleotides and their hybridization properties," *Biochem. Soc. Transact.*, 16:216–217 (1988).
Wada, A., *International Workshop on Automatic and High Speed DNA Base Sequencing*, Hayashibara Forum 1987 at Hayashibara Biochemical Laboratories, Okayama, Japan, Jul. 7–9, 1987.
Walker et al., "Photolabile Protecting Groups for an Acetylcholine Receptor Ligand. Synthesis and Photochemistry of a New Class of o–Nitrobenzyl Derivatives and their Effects on Receptor Function," *Biochemistry*, 25:1799–1805 (1986).
Wallace et al., "The use of synthetic oligonucleotides as hybridization probes. II. Hybridization of oligonucleotides of mixed sequence to rabbit β–globoin DNA," *Nuc. Acids Res.*, 9(4):879 (1981).
Wallace et al., "Hybridization of synthetic oligodeoxyribonucleotides to Φ_x174 DNA: the effect of single base pair mismatch," *Nuc. Acids Res.*, 11(6):3543–3557 (1979).
Washizu et al., "Handling Biological Cells Using a Fluid Integrated Circuit," *IEEE Transactions Industry Applications*, 26(2):352–358 (1990).
Wiedmann, M. et al., "Ligase Chain Reaction (LCR)—Overview and Applications," *PCR Meth. Appl.*, 3(4):S51–S64 (1994).
Werner et al., "Size–Dependent Separation of Proteins Denatured in SDS by Capillary Electrophoresis Using a Replaceable Sieving Matrix," *Anal. Biochem.*, 212:253–258 (1993).
White et al., "An Evaluation of Confocal Versus Conventional Imaging of Biological Structures by Fluorescence Light Microscopy," *J. Cell Biol.*, 105(1):41–48 (1987).
Widacki et al., "Biochemical Differences in Qa–2 Antigens Expressed by Qa–2+,6+ and Qa–2a+,6– Strains. Evidence for Differential Expression of the Q7 and Q9 Genes," *Mol. Immunology*, 27(6):559–570 (1990).
Wilcox et al., "Synthesis of Photolabile 'Precursors' of Amino Acid Neurotransmitters," *J. Org. Chem.*, 55:1585–1589 (1990).
Wilding et al., "PCR in a Silicon Microstructure," *Clin. Chem.*, 40(9):1815–1818 (1994).
Wilding et al., "Manipulation and Flow of Biological Fluids in Straight Channels Micromachined in Silicon," *Clin. Chem.*, 40(1):43–47 (1994).
Wittman–Liebold, eds., Methods in Protein Sequence Analysis, from Proceedings of 7th Int'l Conf., Berlin, Germany, Jul. 3–8, 1988, table of contents, pp. xi–xx* (1989).
Wood et al., "Base composition–independent hybridization in tetramethylammonium chloride: A method for oligonucleotide screening of highly complex gene libraries," *PNAS*, 82:1585–1588 (1985).

Woolley et al., "Ultra-high-speed DNA fragment separations using microfabricated capillary array electrophoresis chips," *PNAS*, 91:11348–11352 (1994).

Wu et al., "Synthesis and Properties of Adenosine-5'-triphosphoro-γ-5-(5-sulfonic acid)naphthyl Ethylamidate: A Fluorescent Nucleotide Substrate for DNA-Dependent RNA Polymerase from *Escherichia coli*," *Arch. Biochem. Biophys.*, 246(2):564–571 (1986).

Wu et al., "Laboratory Methods, Direct Analysis of Single Nucleotide Variation in Human DNA and RNA Using In Situ Dot Hybridization," *DNA*, 8(2):135–142 (1989).

Yamamoto et al., "Features and applications of the laser scanning microscope," *J. Mod. Optics*, 37(11):1691–1701 (1990).

Yarbrough et al., "Synthesis and Properties of Fluorescent Nucleotide Substrates for DNA-dependent RNA Polymerases," *J. Biol. Chem.*, 254(23):12069–12073 (1979).

Yosomiya et al., "Performance, Glass fiber Having Isocyanate Group on the Surface. Preparation and Reaction with Amino Acid," *Polymer Bulletin*, 12:41–48 (1984).

Young, W.S., "Simultaneous Use of Digoxigenin-and Radiolabeled Oligodeoxyribonucleotide Probes for Hybridization Histochemistry," *Neuropeptides*, 13:271–275 (1989).

Yue et al., "Miniature Field-Flow Fractionation System for Analysis of Blood Cells," *Clin.Chem.*, 40(9):1810–1814 (1994).

Zehavi et al., "Light-Sensitive Glycosides: I. 6-Nitroveratryl β-D-Glucopyranoside and 2-Nitrobenzyl β-D-Glucopyranoside," *J. Org. Chem.*, 37(14):2281–2285 (1972).

Zengerle et al., "Transient measurements on miniaturized diaphragm pumps in microfluid systems," *Sensors and Actuators*, A46-47:557–561 (1995).

Zischler et al., "Non-radioacive oligonucleotide fingerprinting in the gel," *Nuc. Acids Res.*, 17(11)4411 (1989).

Zischler et al., "Digoxigenated oligonucleotide probes specific for simple repeats in DNA fingerprinting and hybridization in situ," *Hum. Genet.*, 82:227–233 (1989).

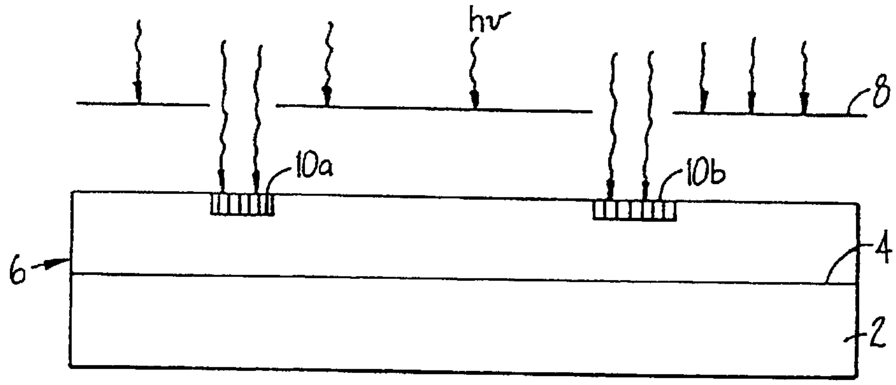


FIG._1.

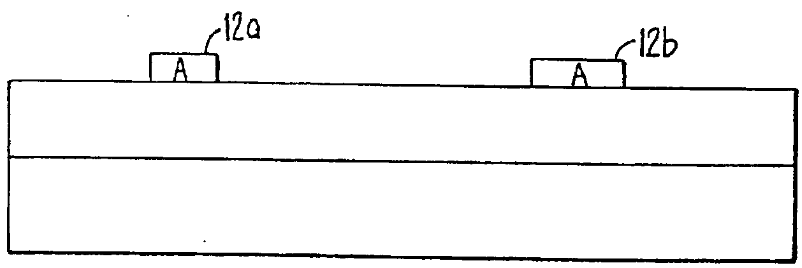


FIG._2.

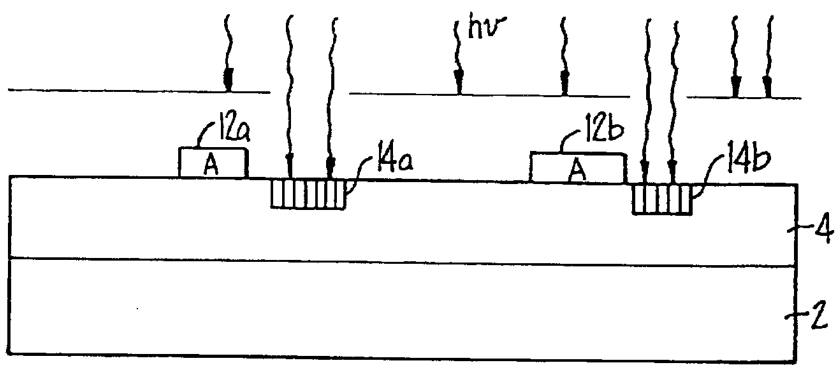


FIG._3.

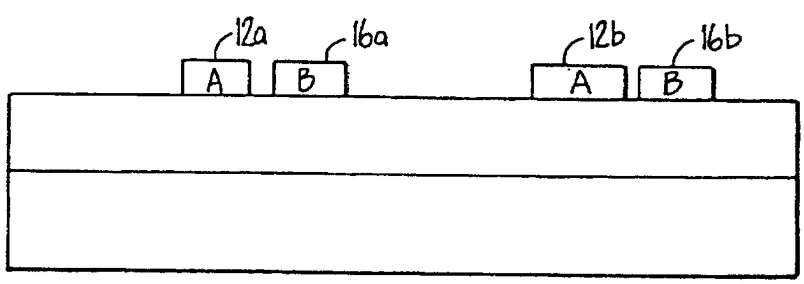


FIG._4.

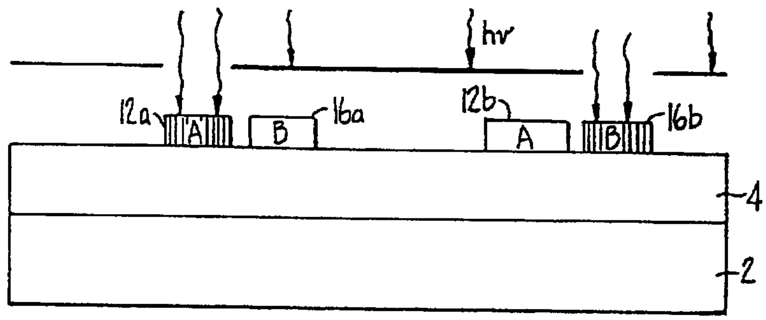


FIG._5.

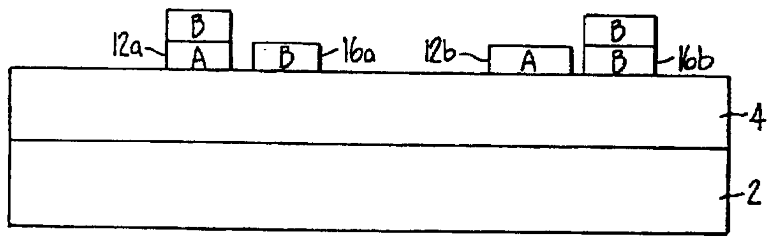


FIG._6.

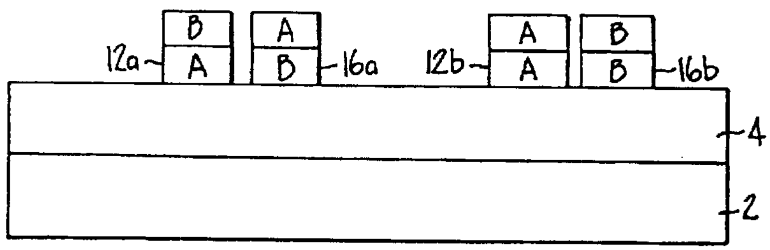


FIG._7.

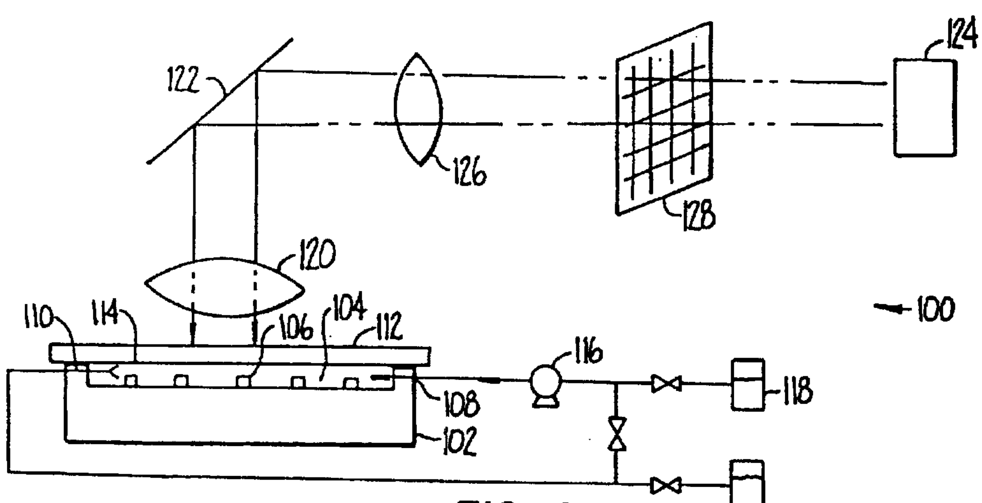


FIG._8a.

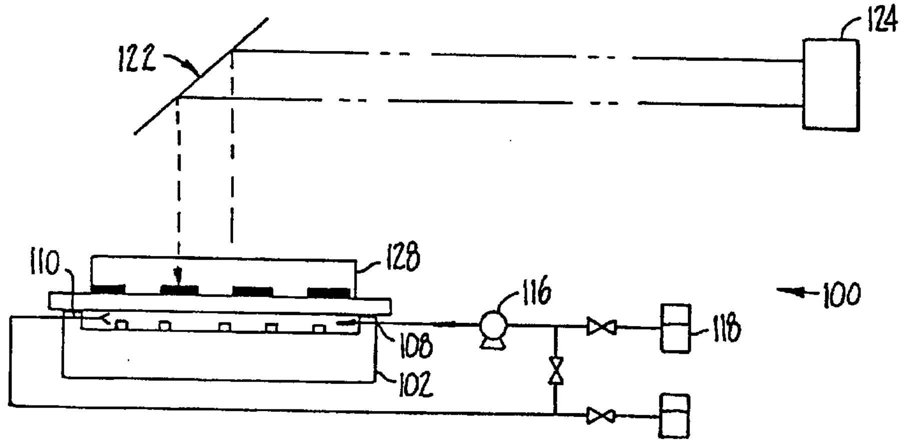


FIG._8b.

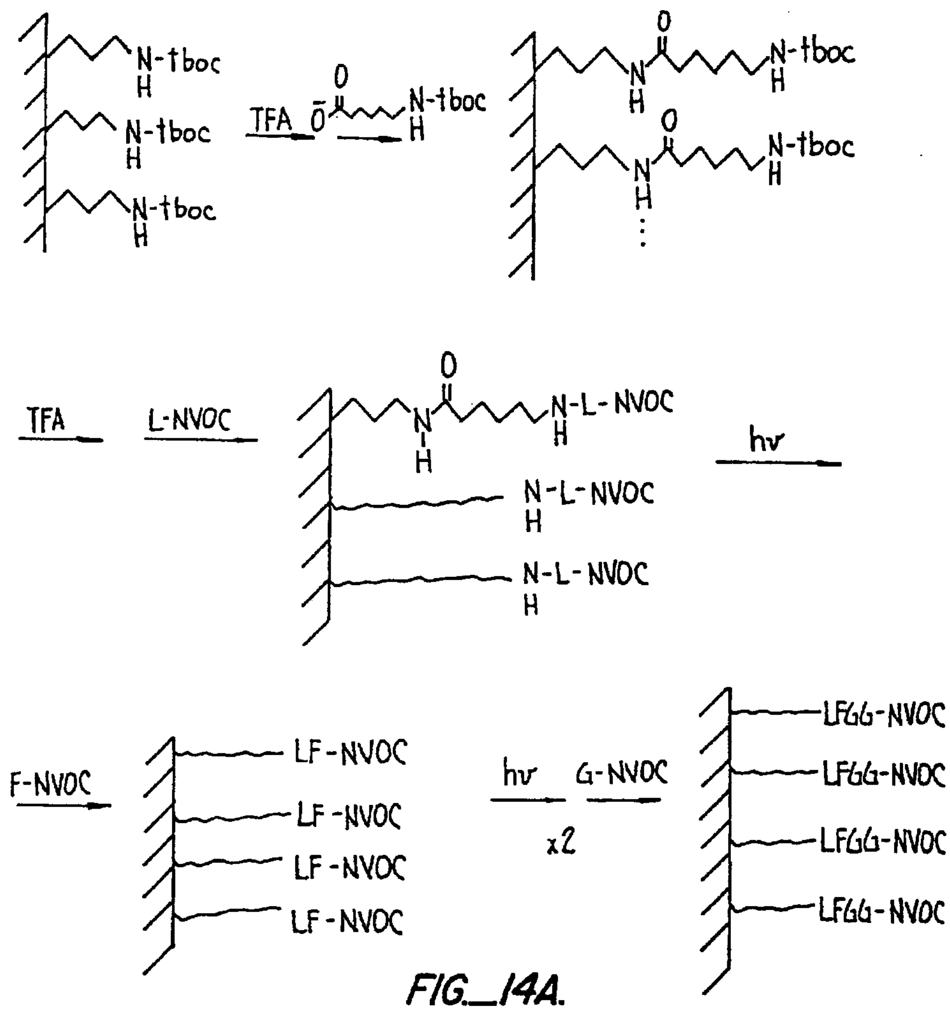


FIG._14A.

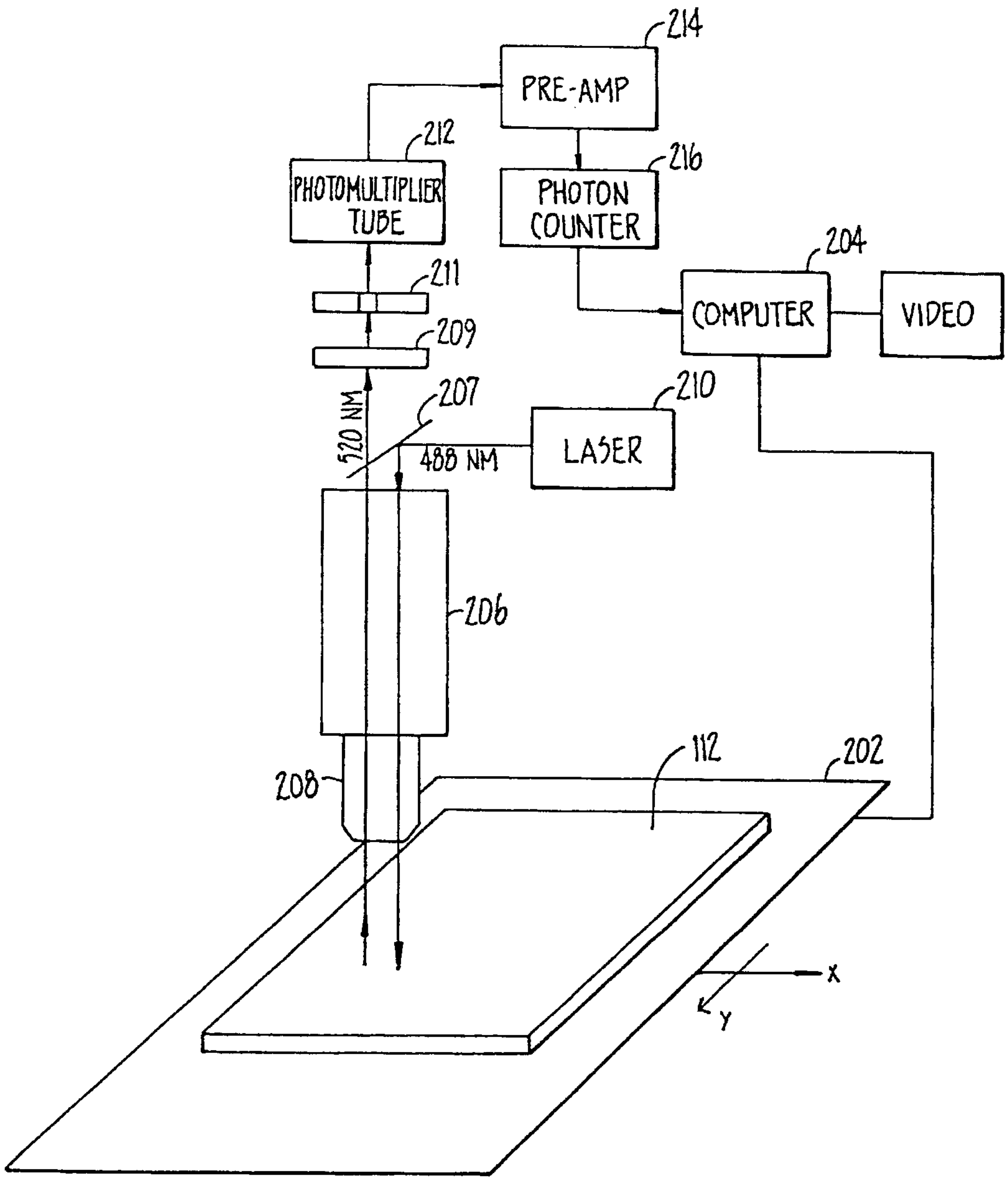

214
PRE-AMP
212
PHOTOMULTIPLIER TUBE
216
PHOTON COUNTER
204
COMPUTER
VIDEO
211
209
207
520 NM
488 NM
210
LASER
206
112
202
208
X
Y

FIG._9.

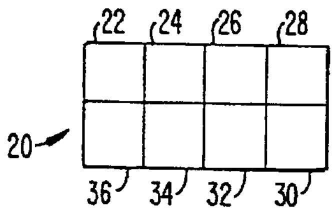


FIG._10A.

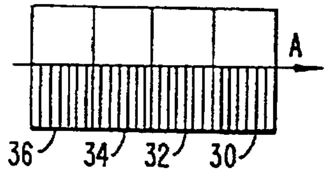


FIG._10B.

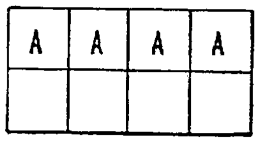


FIG._10C.

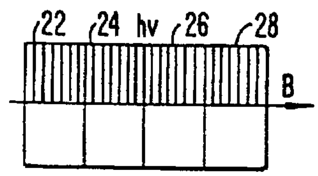


FIG._10D.

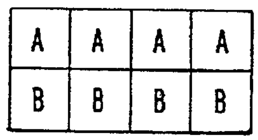


FIG._10E.

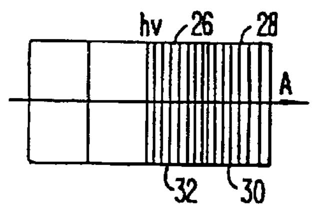


FIG._10F.

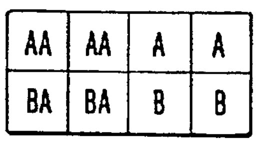


FIG._10G.

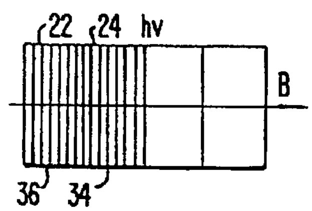


FIG._10H.

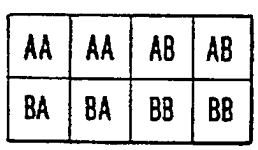


FIG._10I.

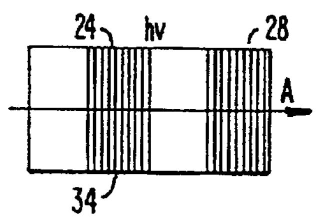


FIG._10J.

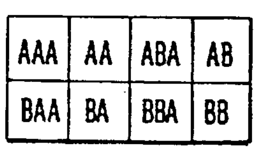


FIG._10K.

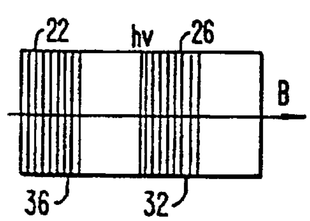


FIG._10L.

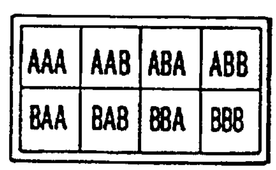


FIG._10M.

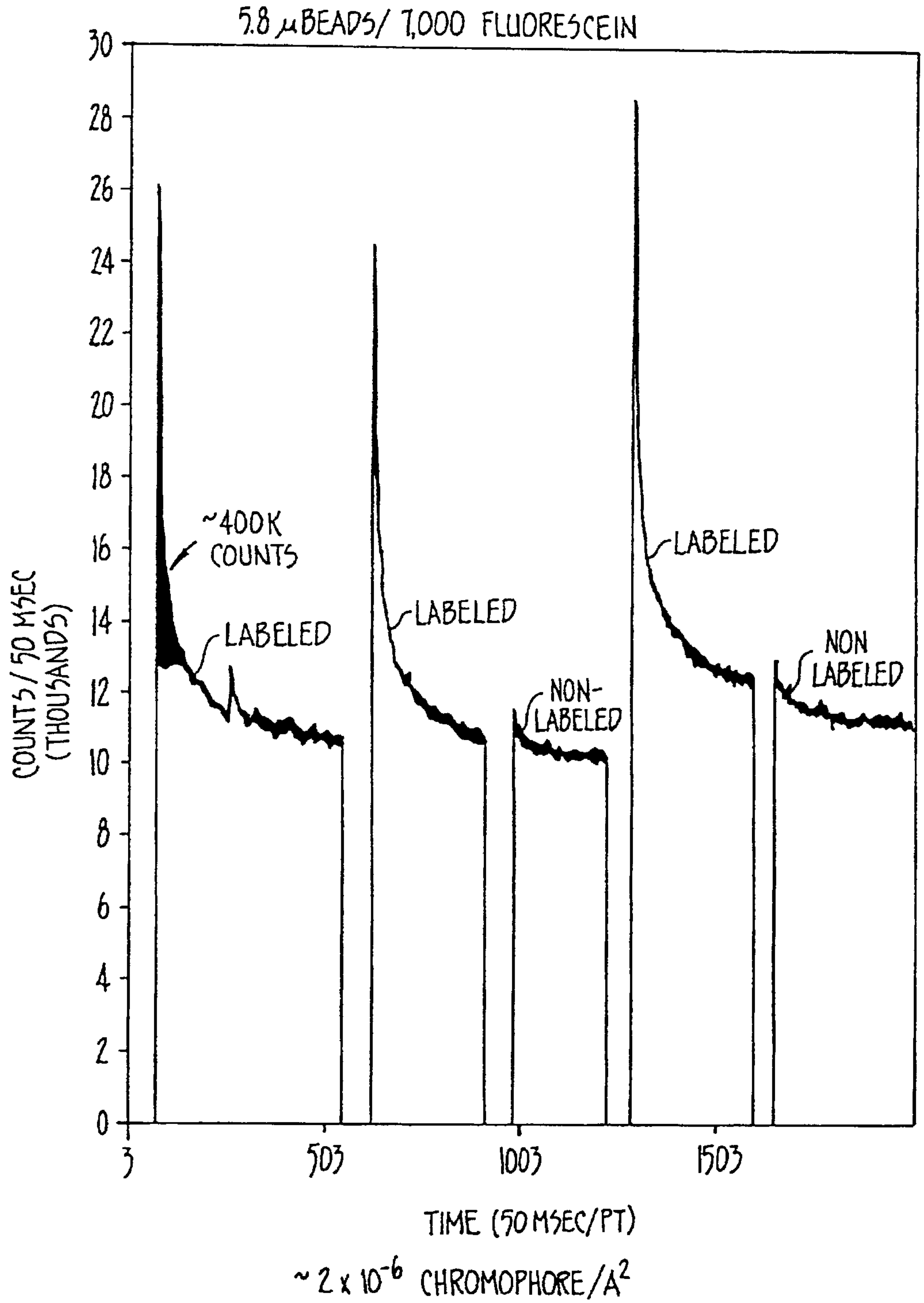

5.8 μ BEADS/ 7,000 FLUORESCEIN
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
COUNTS / 50 MSEC
(THOUSANDS)
~400K
COUNTS
LABELED
LABELED
NON-
LABELED
LABELED
NON
LABELED
3
503
1003
1503
TIME (50 MSEC/PT)
~ 2 x 10^-6 CHROMOPHORE/A^2

FIG._11A.

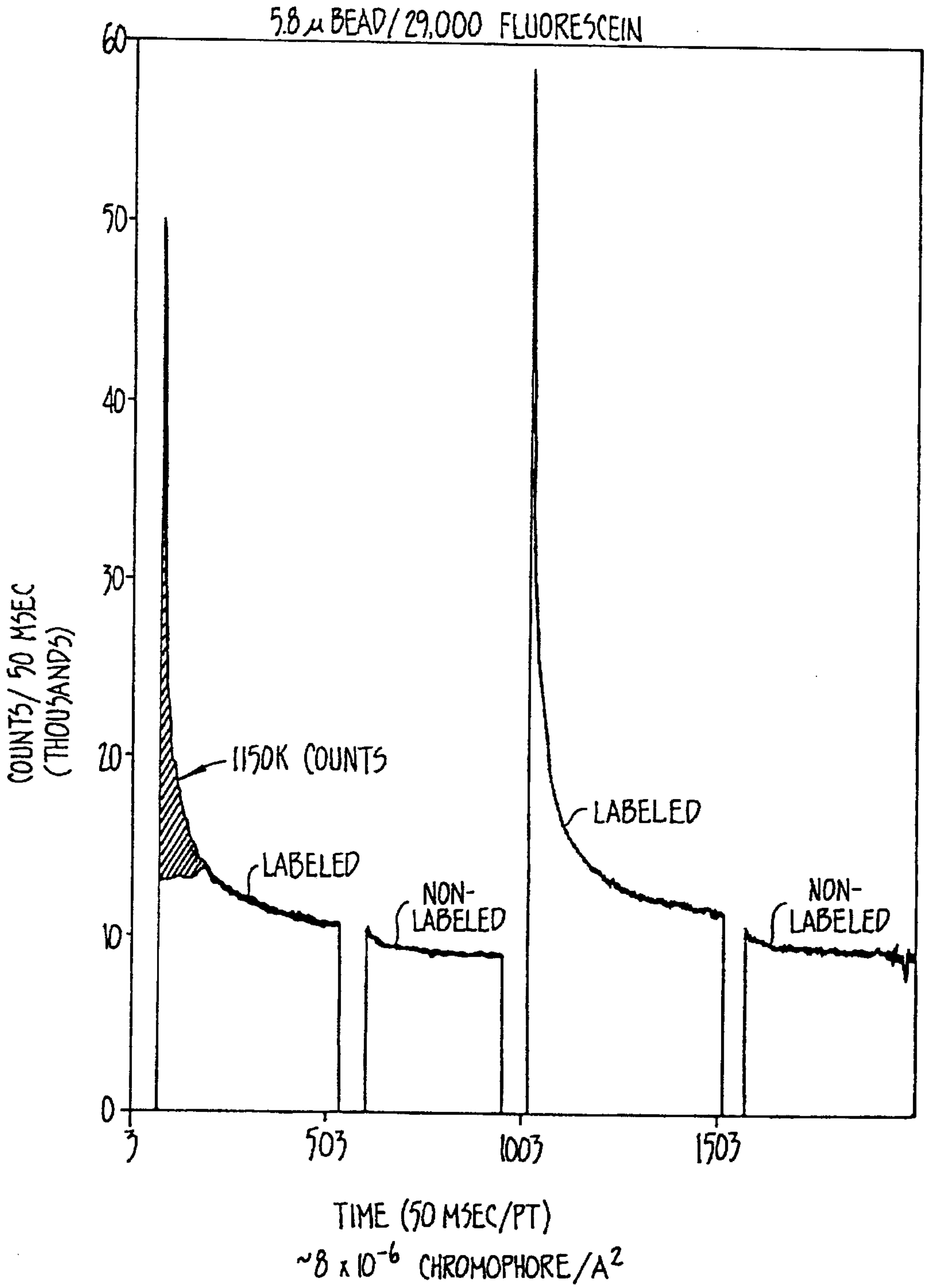

5.8 μ BEAD/29,000 FLUORESCEIN
COUNTS/50 MSEC (THOUSANDS)
60
50
40
30
20
10
0
1150K COUNTS
LABELED
NON-LABELED
LABELED
NON-LABELED
3
503
1003
1503
TIME (50 MSEC/PT)
~8 x 10^-6 CHROMOPHORE/A^2

FIG._11B.

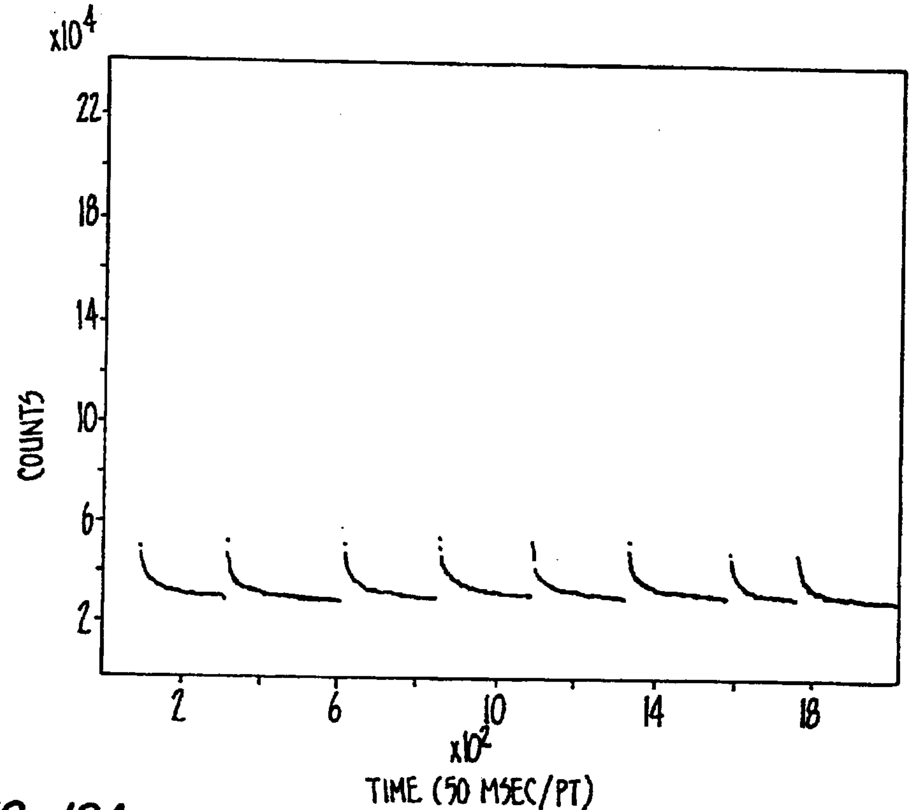


FIG_12A.

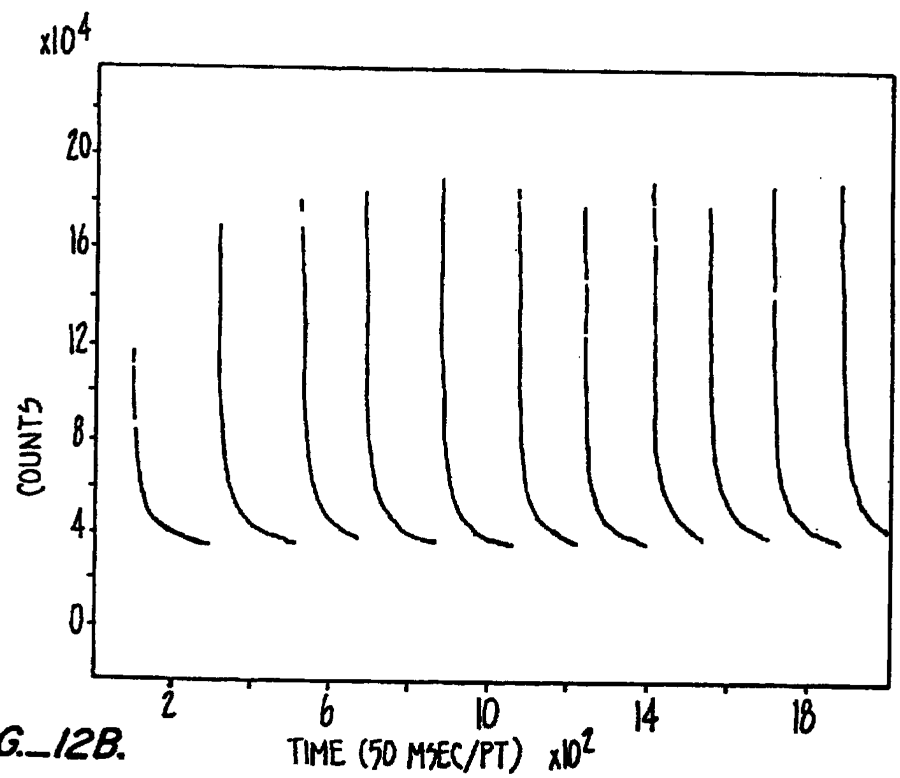
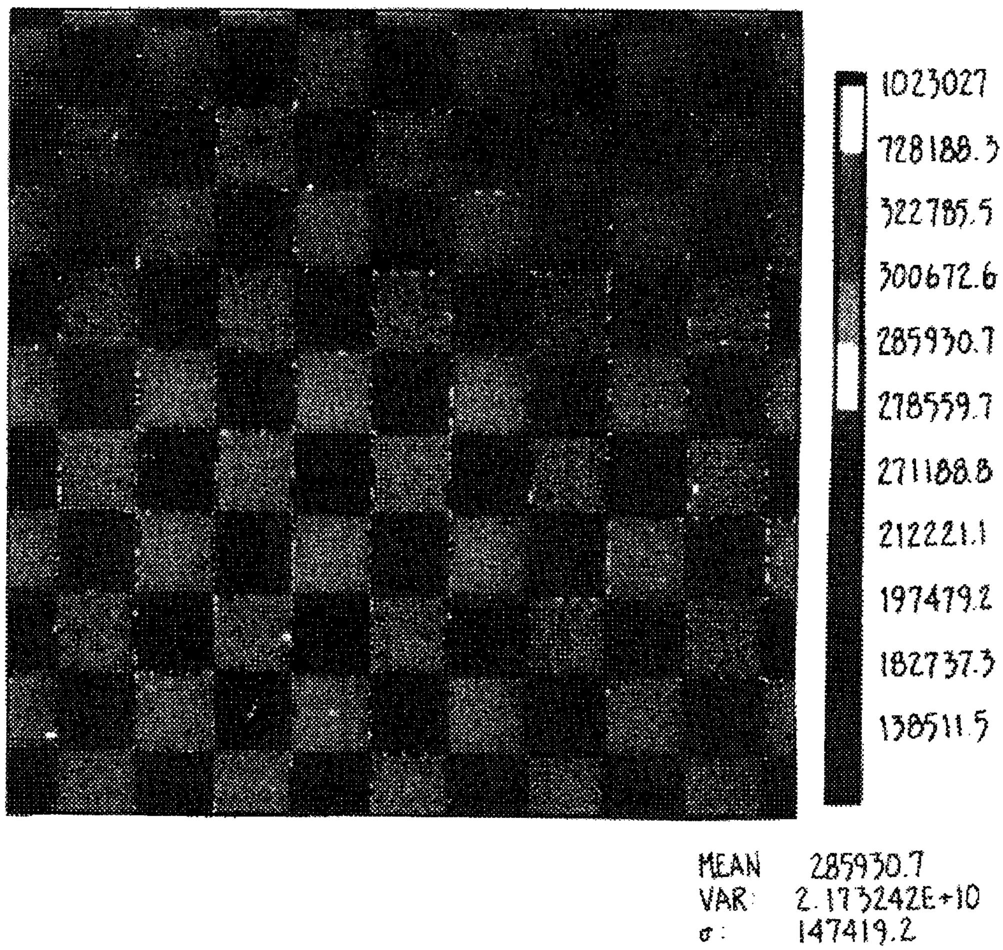


FIG_12B.



1023027
728188.3
322785.5
300672.6
285930.7
278559.7
271188.8
212221.1
197479.2
182737.3
138511.5
MEAN 285930.7
VAR: 2.173242E+10
σ: 147419.2

FIG._13A.

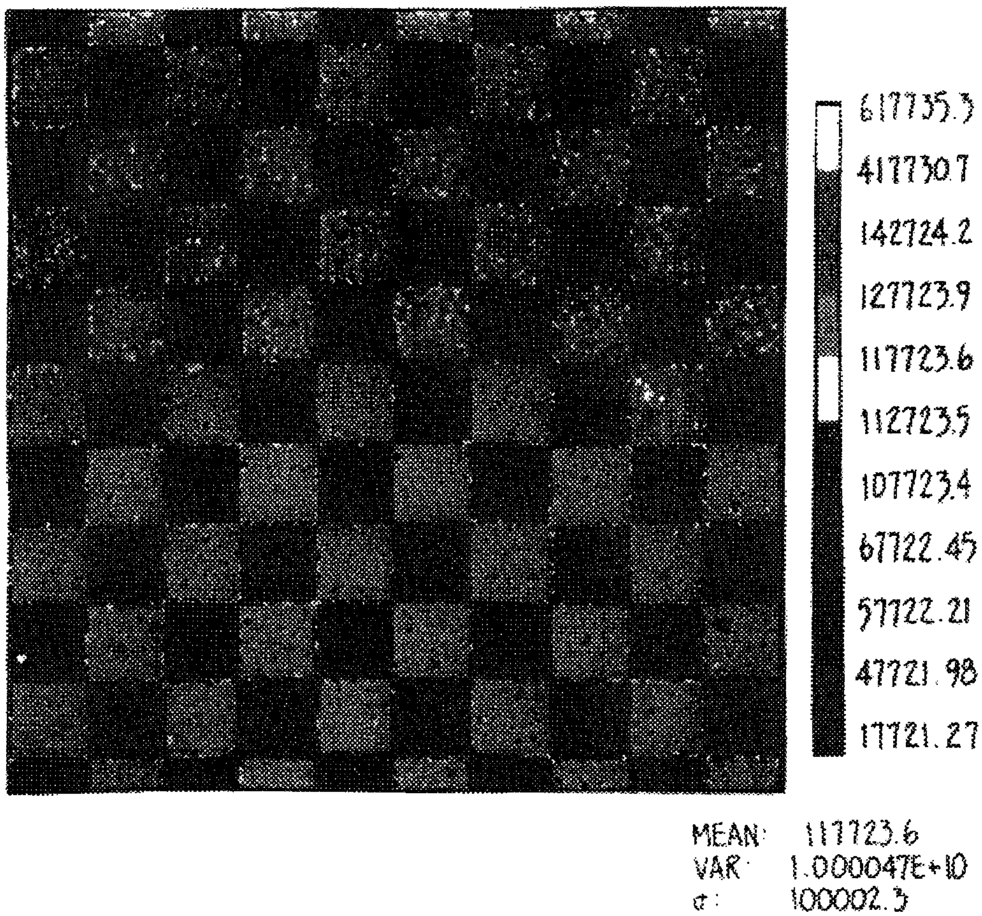

617735.3
417730.7
142724.2
127723.9
117723.6
112723.5
107723.4
67722.45
57722.21
47721.98
17721.27
MEAN: 117723.6
VAR: 1.000047E+10
σ: 100002.3

FIG._13B.

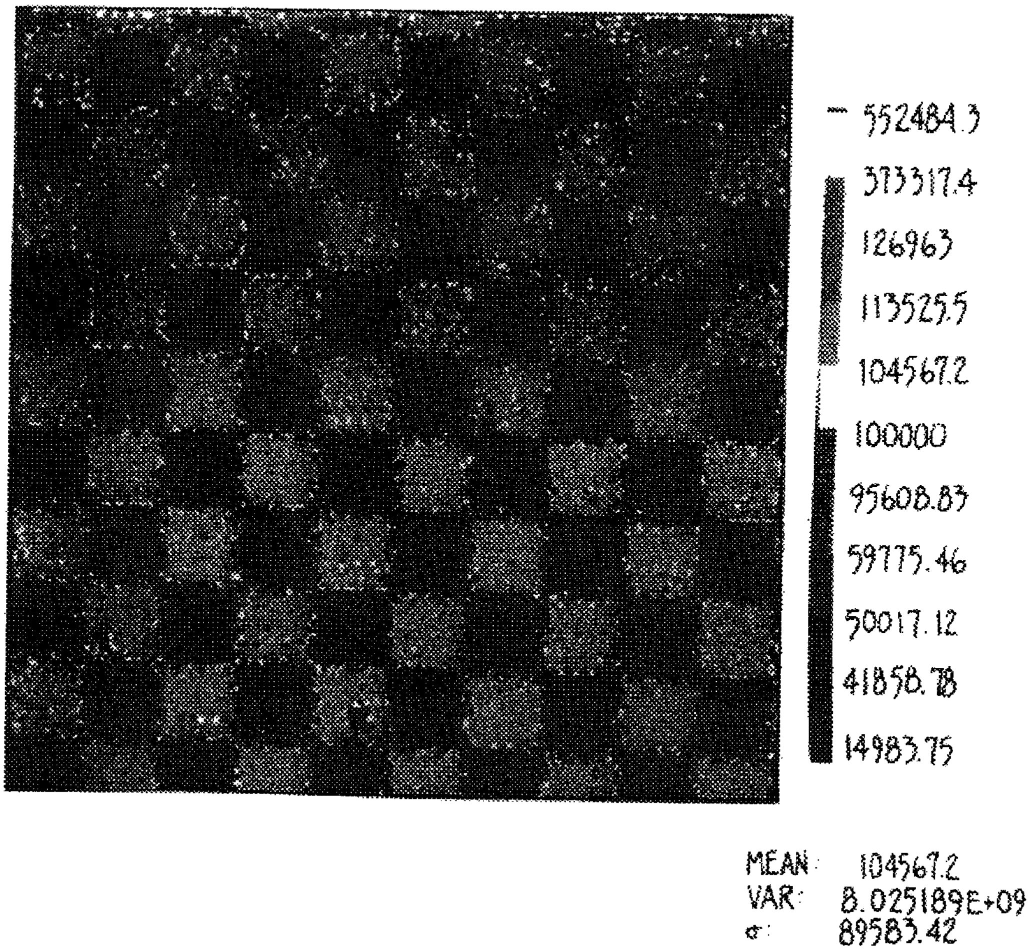

552484.3
373317.4
126963
113525.5
104567.2
100000
95608.83
59775.46
50017.12
41858.78
14983.75
MEAN: 104567.2
VAR: 8.025189E+09
σ: 89583.42

FIG._13C.

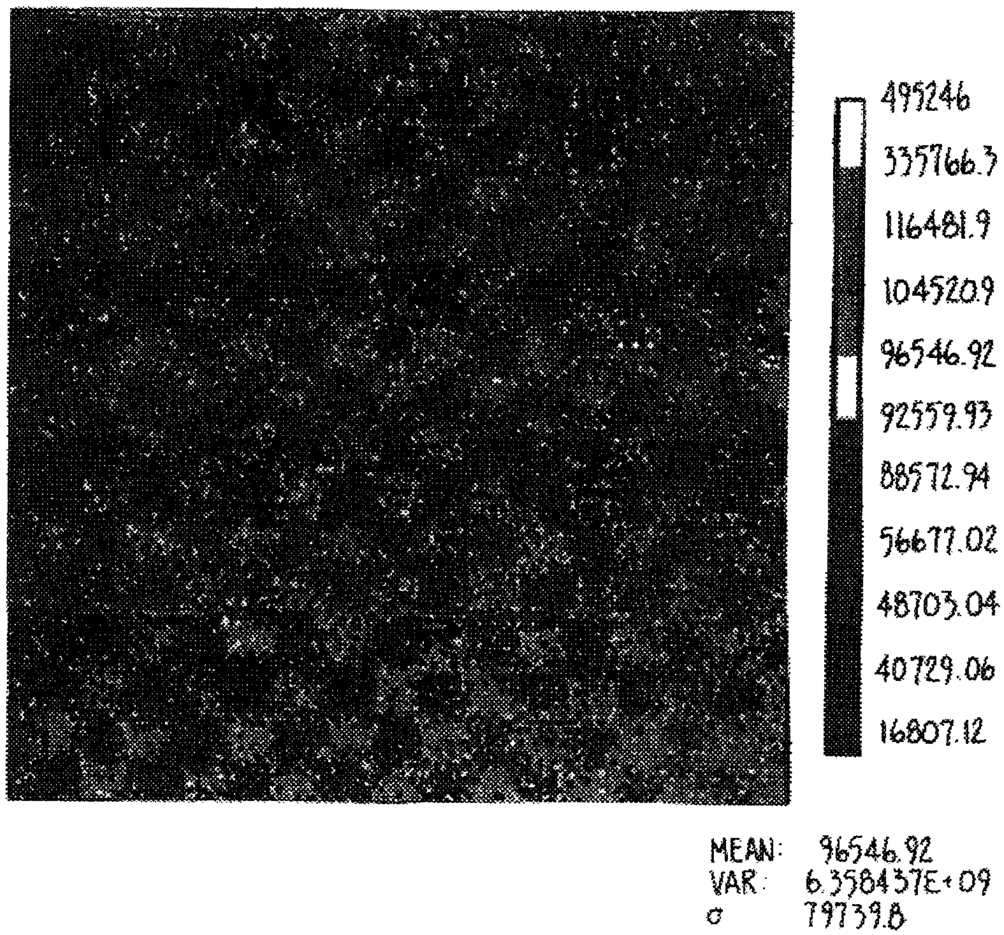
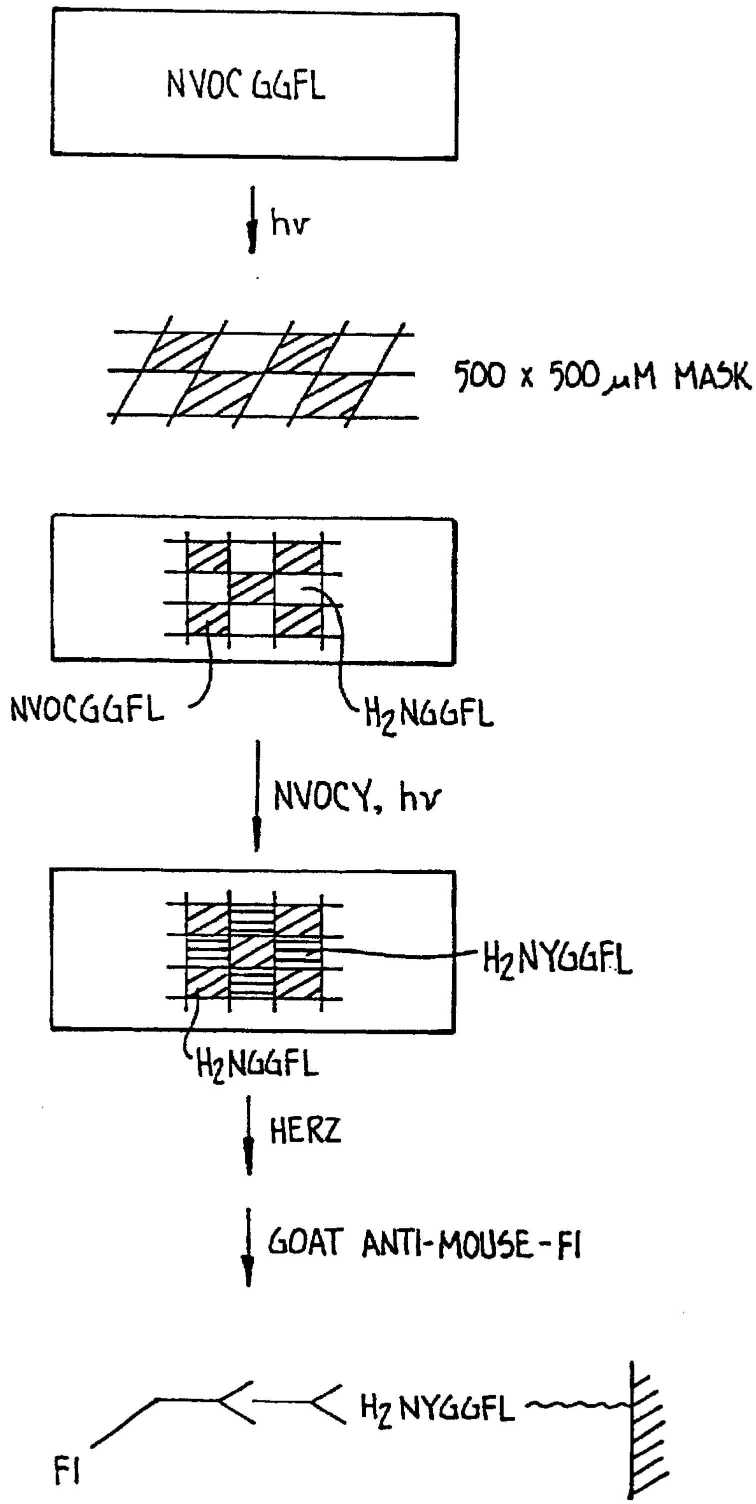

495246
335766.3
116481.9
104520.9
96546.92
92559.93
88572.94
56677.02
48703.04
40729.06
16807.12
MEAN: 96546.92
VAR: 6.358437E+09
σ 79739.8

FIG._13D.



NVOC GGFL
hv
500 x 500 μM MASK
NVOCGGFL
H2NGGFL
NVOCY, hv
H2NYGGFL
H2NGGFL
HERZ
GOAT ANTI-MOUSE-FI
FI
H2NYGGFL

FIG._14B.

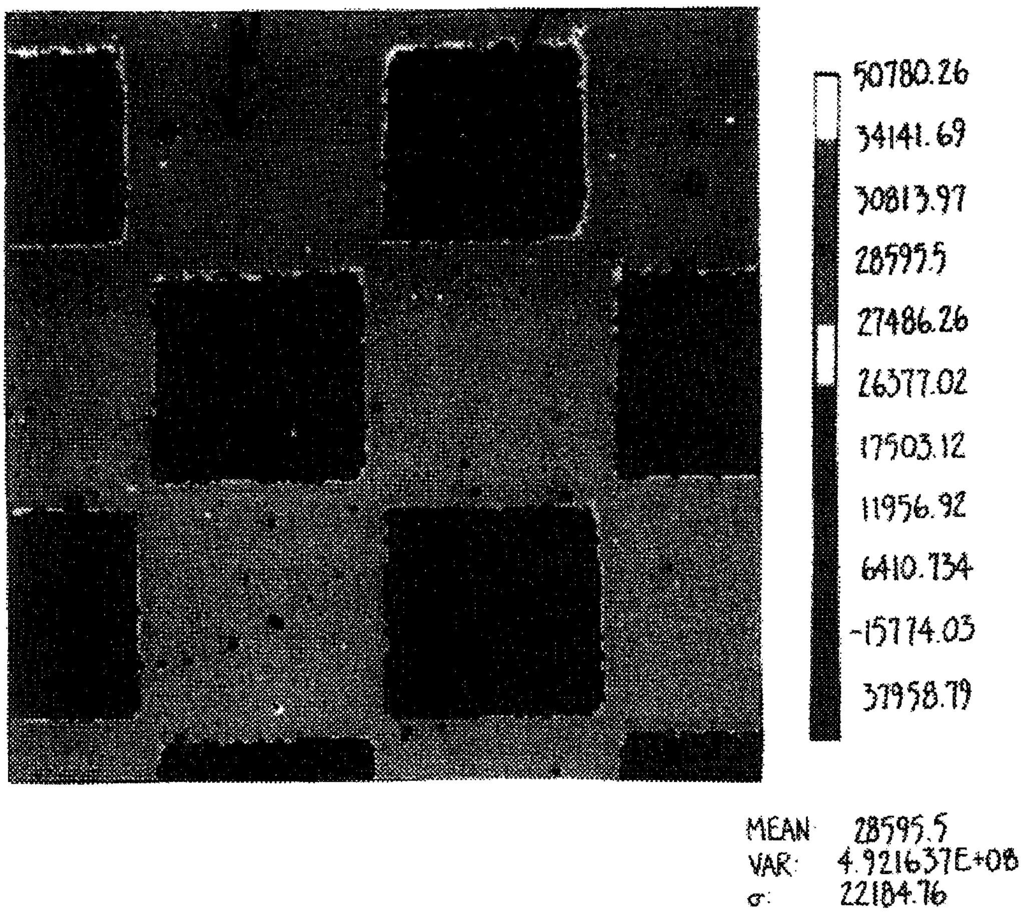

50780.26
34141.69
30813.97
28595.5
27486.26
26377.02
17503.12
11956.92
6410.734
-15774.03
37958.79
MEAN 28595.5
VAR: 4.921637E+08
σ: 22184.76

FIG._15A.

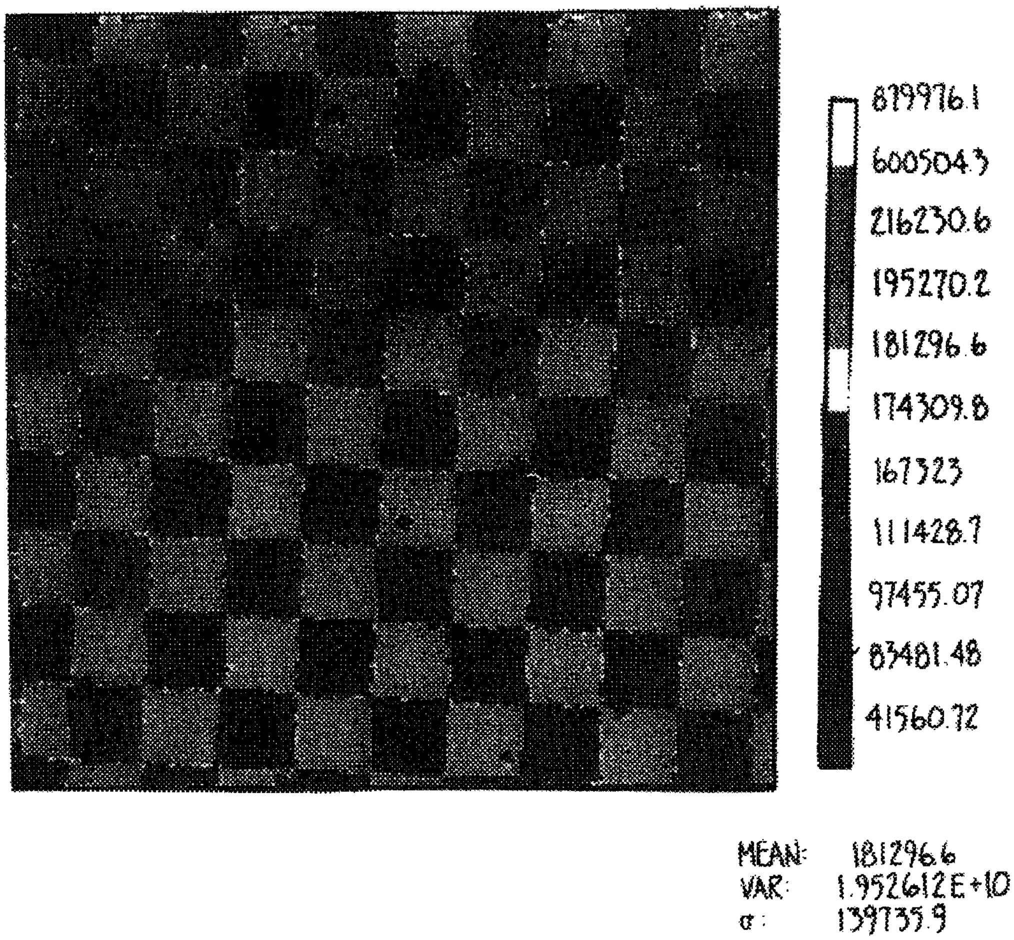

879976.1
600504.3
216230.6
195270.2
181296.6
174309.8
167323
111428.7
97455.07
83481.48
41560.72
MEAN: 181296.6
VAR: 1.952612E+10
σ: 139735.9

FIG._15B.

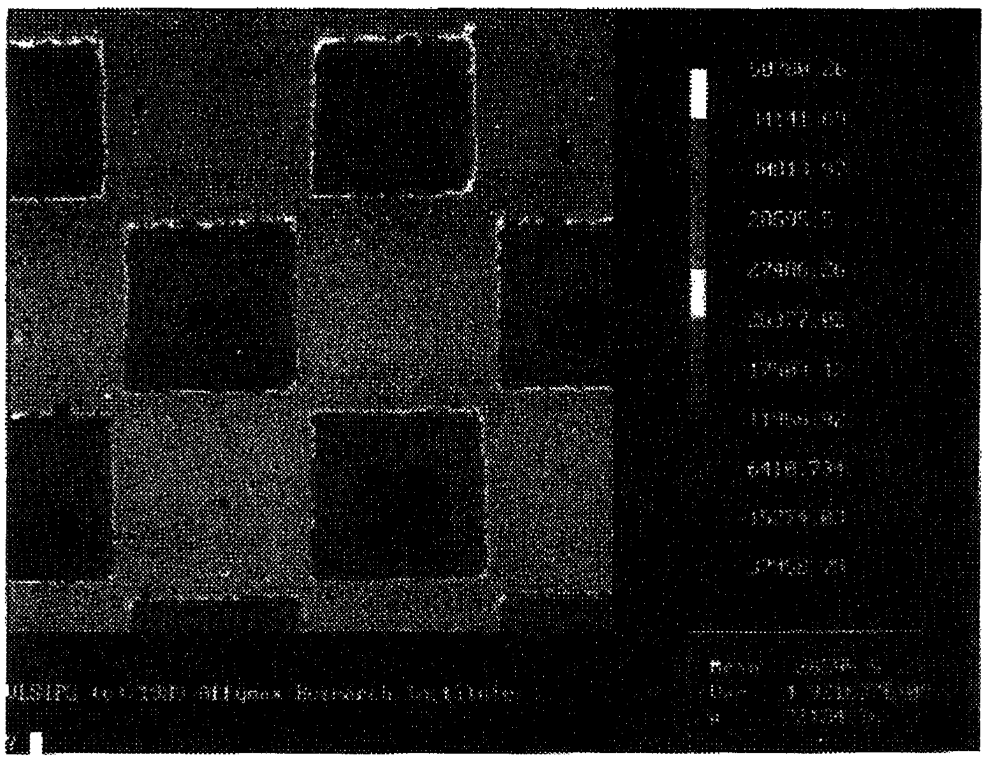


FIG. 15C

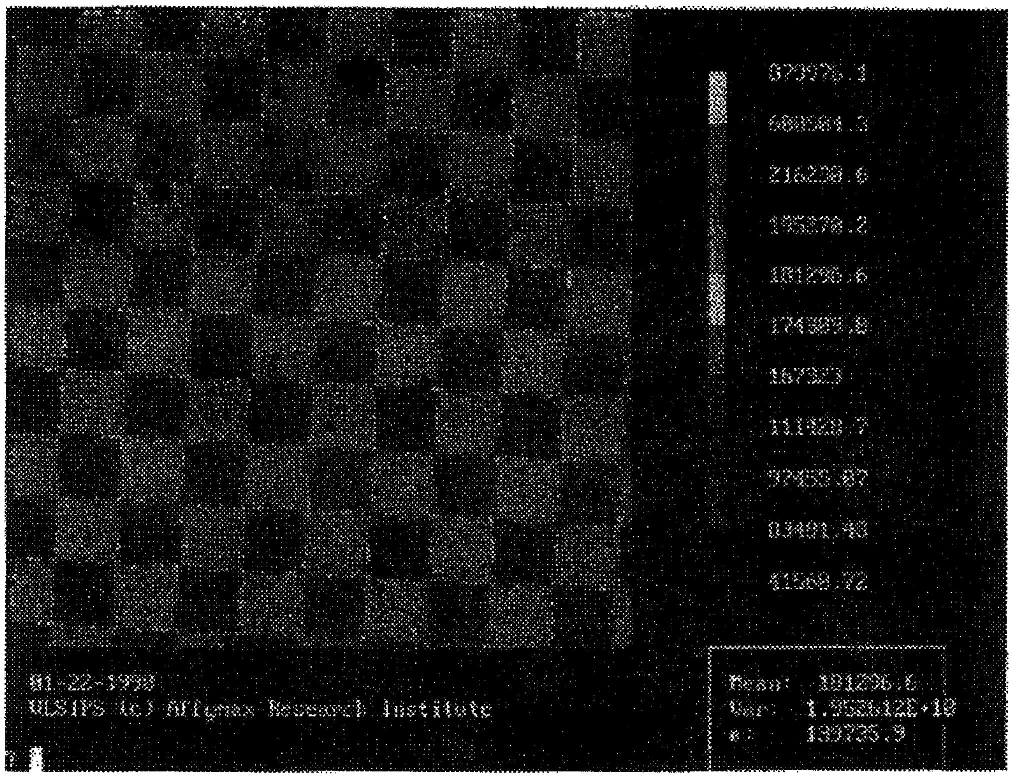

167323
01-22-1998
VLSIPS (c) Affymax Research Institute
Mean: 181296.6

FIG. 15D

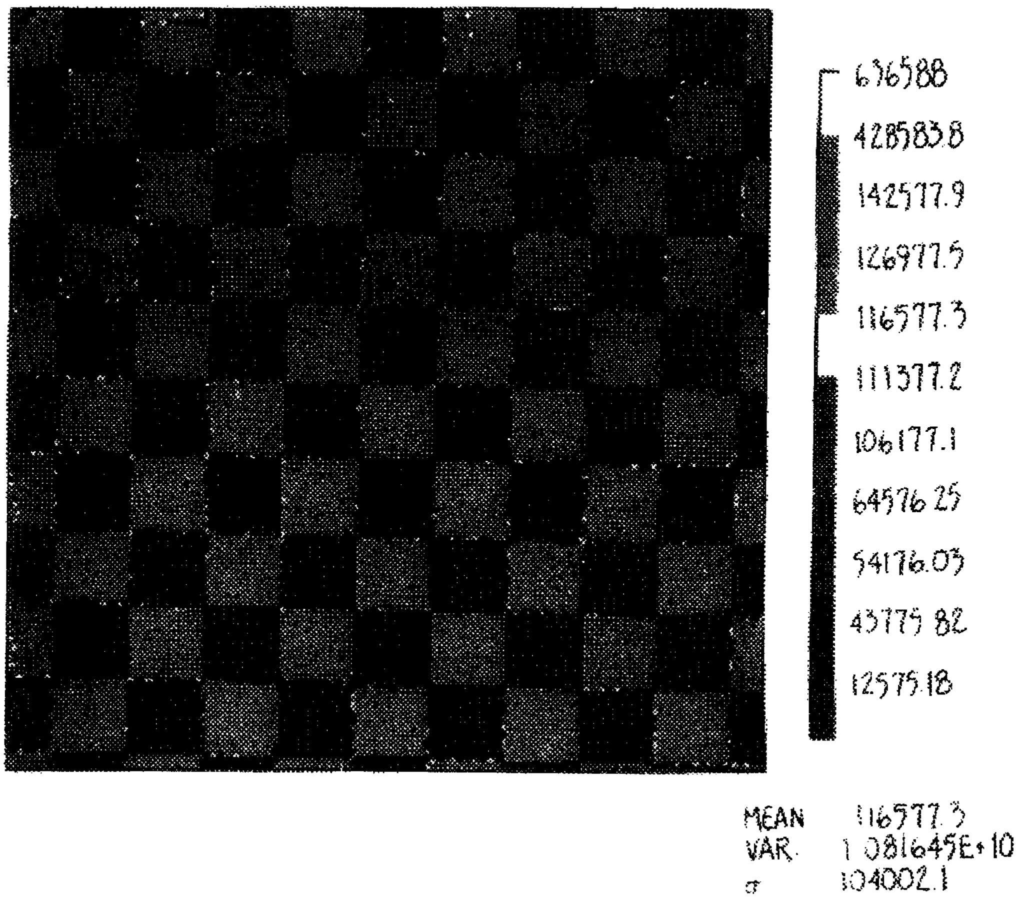
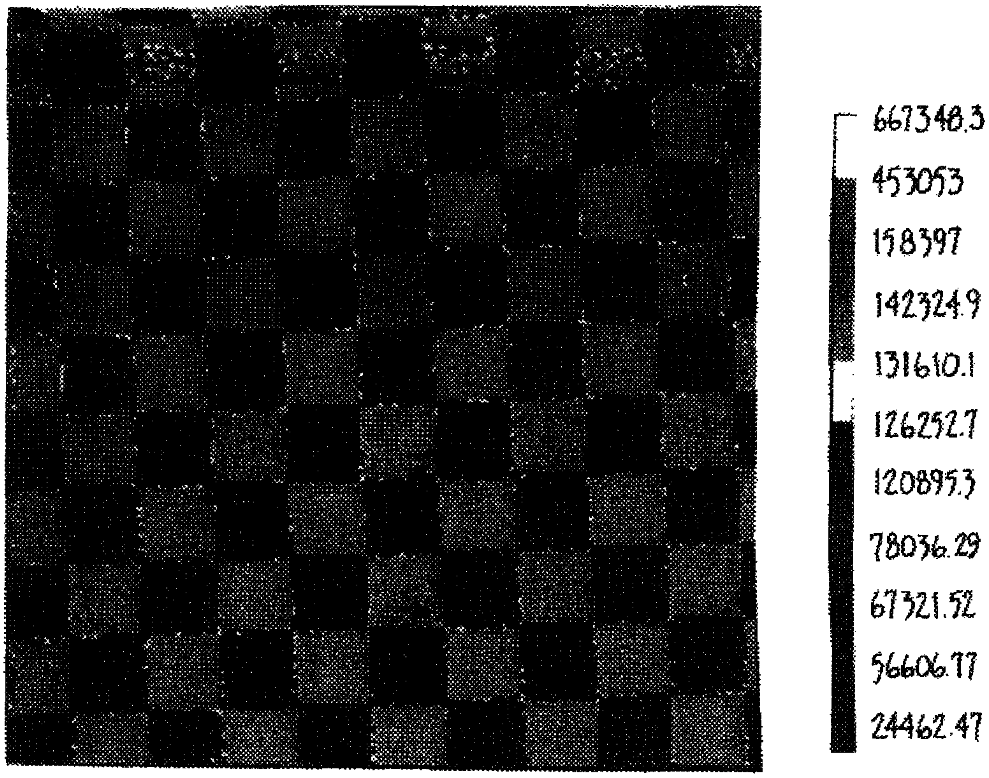

636588
428583.8
142577.9
126977.5
116577.3
111377.2
106177.1
64576.25
54176.03
43775.82
12575.18
MEAN 116577.3
VAR 1.081645E+10
σ 104002.1

FIG._16.



MEAN: 131610.1
VAR: 1.148062E+10
σ: 107147.6

FIG._17.

P	A	S	G	
LPGFL	LAGFL	LSGFL	LGGFL	L
FPGFL	FAGFL	FSGFL	FGGFL	F
WPGFL	WAGFL	WSGFL	WGGFL	W
YPGFL	YAGFL	YSGFL	YGGFL	Y

L SET

FIG._18A.

p	a	s	G	
YpGFL	YaGFL	YsGFL	YGGFL	Y
fpGFL	faGFL	fsGFL	fGGFL	f
wpGFL	waGFL	wsGFL	wGGFL	w
ypGFL	yaGFL	ysGFL	yGGFL	y

D SET

FIG._18B.

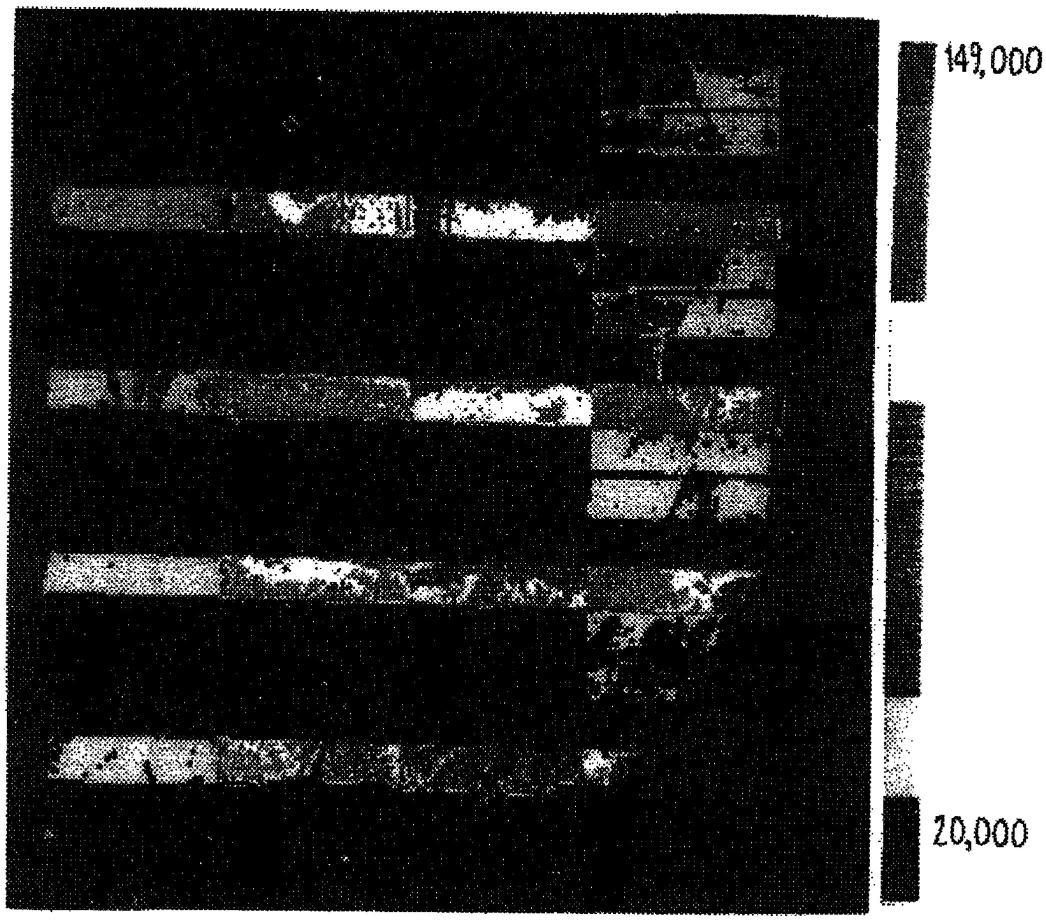


FIG._19.

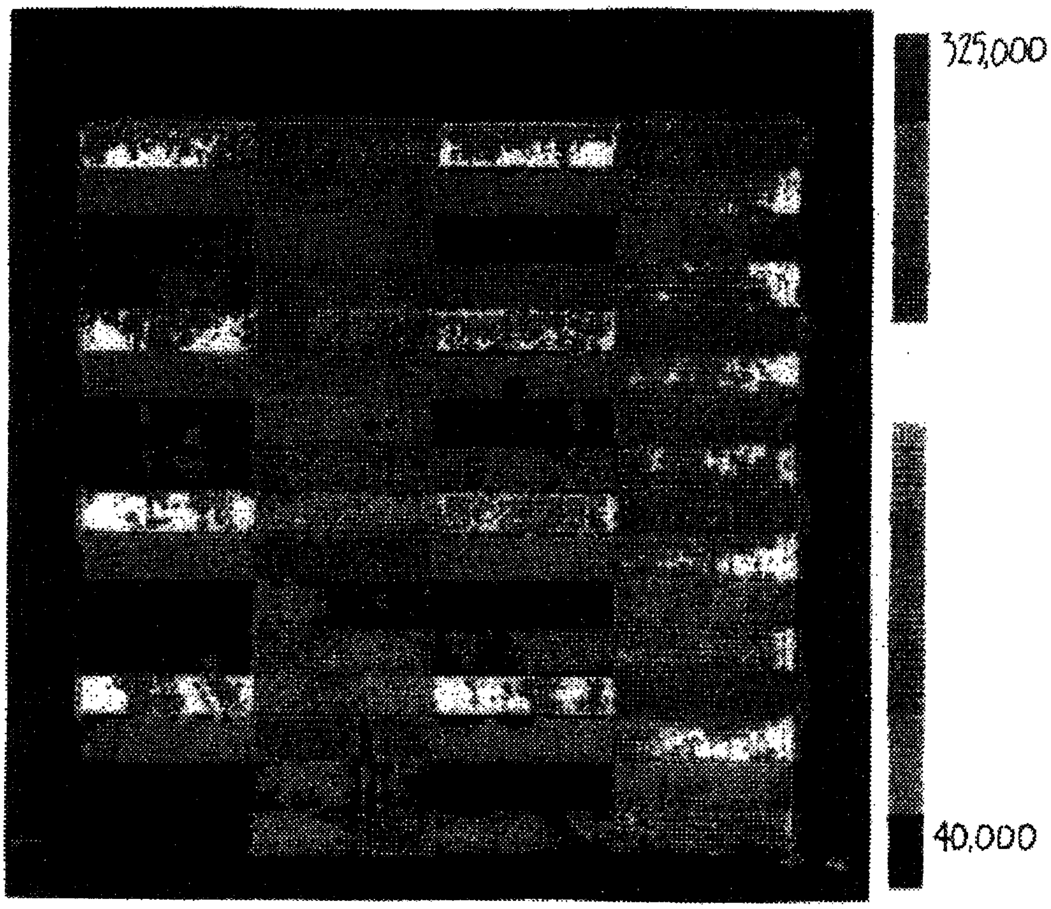


FIG._20

NUCLEIC ACID READING AND ANALYSIS SYSTEM

The present application is a continuation of and claims priority to 09/690,191 filed Oct. 16, 2000 now U.S. Pat. No. 6,403,957 which is a continuation of 09/129,470 filed Aug. 4, 1998 (U.S. Pat. No. 6,329,143) which is a continuation of 08/456,598 filed Jun. 1, 1995 (U.S. Pat. No. 6,225,625), which is a divisional of 07/954,646 filed Sep. 30, 1992 (U.S. Pat. No. 5,445,934), which is a divisional of 07/850,356 filed Mar. 12, 1992 (U.S. Pat. No. 5,405,783) which is a divisional of 07/492,462 filed Mar. 7, 1990 (U.S. Pat. No. 5,143,854), which is a continuation-in-part of 07/362,901 filed Jun. 7, 1989, now abandoned, the disclosures of which are incorporated by reference.

COPYRIGHT NOTICE

BACKGROUND OF THE INVENTION

The present inventions relate to the synthesis and placement materials at known locations. In particular, one embodiment of the inventions provides a method and associated apparatus for preparing diverse chemical sequences at known locations on a single substrate surface. The inventions may be applied, for example, in the field of preparation of oligomer, peptide, nucleic acid, oligosaccharide, phospholipid, polymer, or drug congener preparation, especially to create sources of chemical diversity for use in screening for biological activity.

The relationship between structure and activity of molecules is a fundamental issue in the study of biological systems. Structure-activity relationships are important in understanding, for example, the function of enzymes, the ways in which cells communicate with each other, as well as cellular control and feedback systems.

Certain macromolecules are known to interact and bind to other molecules having a very specific three-dimensional spatial and electronic distribution. Any large molecule having such specificity can be considered a receptor, whether it is an enzyme catalyzing hydrolysis of a metabolic intermediate, a cell-surface protein mediating membrane transport of ions, a glycoprotein serving to identify a particular cell to its neighbors, an IgG-class antibody circulating in the plasma, an oligonucleotide sequence of DNA in the nucleus, or the like. The various molecules which receptors selectively bind are known as ligands.

Many assays are available for measuring the binding affinity of known receptors and ligands, but the information which can be gained from such experiments is often limited by the number and type of ligands which are available. Novel ligands are sometimes discovered by chance or by application of new techniques for the elucidation of molecular structure, including x-ray crystallographic analysis and recombinant genetic techniques for proteins.

Small peptides are an exemplary system for exploring the relationship between structure and function in biology. A peptide is a sequence of amino acids. When the twenty naturally occurring amino acids are condensed into polymeric molecules they form a wide variety of three-

dimensional configurations, each resulting from a particular amino acid sequence and solvent condition. The number of possible pentapeptides of the 20 naturally occurring amino acids, for example, is 20^5 or 3.2 million different peptides. The likelihood that molecules of this size might be useful in receptor-binding studies is supported by epitope analysis studies showing that some antibodies recognize sequences as short as a few amino acids with high specificity. Furthermore, the average molecular weight of amino acids puts small peptides in the size range of many currently useful pharmaceutical products.

Pharmaceutical drug discovery is one type of research which relies on such a study of structure-activity relationships. In most cases, contemporary pharmaceutical research can be described as the process of discovering novel ligands with desirable patterns of specificity for biologically important receptors. Another example is research to discover new compounds for use in agriculture, such as pesticides and herbicides.

Sometimes, the solution to a rational process of designing ligands is difficult or unyielding. Prior methods of preparing large numbers of different polymers have been painstakingly slow when used at a scale sufficient to permit effective rational or random screening. For example, the "Merrifield" method (*J. Am. Chem. Soc.* (1963) 85:2149–2154, which is incorporated herein by reference for all purposes) has been used to synthesize peptides on a solid support. In the Merrifield method, an amino acid is covalently bonded to a support made of an insoluble polymer. Another amino acid with an alpha protected group is reacted with the covalently bonded amino acid to form a dipeptide. After washing, the protective group is removed and a third amino acid with an alpha protective group is added to the dipeptide. This process is continued until a peptide of a desired length and sequence is obtained. Using the Merrifield method, it is not economically practical to synthesize more than a handful of peptide sequences in a day.

To synthesize larger numbers of polymer sequences, it has also been proposed to use a series of reaction vessels for polymer synthesis. For example, a tubular reactor system may be used to synthesize a linear polymer on a solid phase support by automated sequential addition of reagents. This method still does not enable the synthesis of a sufficiently large number of polymer sequences for effective economical screening.

Methods of preparing a plurality of polymer sequences are also known in which a foraminous container encloses a known quantity of reactive particles, the particles being larger in size than foramina of the container. The containers may be selectively reacted with desired materials to synthesize desired sequences of product molecules. As with other methods known in the art, this method cannot practically be used to synthesize a sufficient variety of polypeptides for effective screening.

Other techniques have also been described. These methods include the synthesis of peptides on 96 plastic pins which fit the format of standard microtiter plates. Unfortunately, while these techniques have been somewhat useful, substantial problems remain. For example, these methods continue to be limited in the diversity of sequences which can be economically synthesized and screened.

From the above, it is seen that an improved method and apparatus for synthesizing a variety of chemical sequences at known locations is desired.

SUMMARY OF THE INVENTION

An improved method and apparatus for the preparation of a variety of polymers is disclosed.